

HLA-A PURIFICATION AND PEPTIDE ELUTION

Cellular Source: HLA-Antigens
($5 \cdot 10 \times 10^6$ cell equivalents)

- a) EBV transformed B cell lines - homozygous
- b) HLA-A transfectants - e.g. .221-HLA-A1
- c) P815 transfectants (mouse mastocytoma)

Detergent Lysis
(10^6 cells/ml)

1% NP-40 or 1% Renex 30 plus
protease inhibitors - 1 hr, 4 C

Detergent Lysate

Centrifugation at 15,000xg,
30 min.

Affinity Chromatography

mAb-Sepharose 5 mg/ml
5-10 ml column

Purified HLA-A Antigen

Anticipated yields 450-900 μ g

Acid Treatment

10% acetic acid, 5 min, 100°C

Peptides

YM3 filtration, 3kD cut-off

Sequence/Motif

D. Hunt - HPLC/EI-TMS
Cytel - HPLC/ABI 477A

0065510-091900

006T60" 0T559960

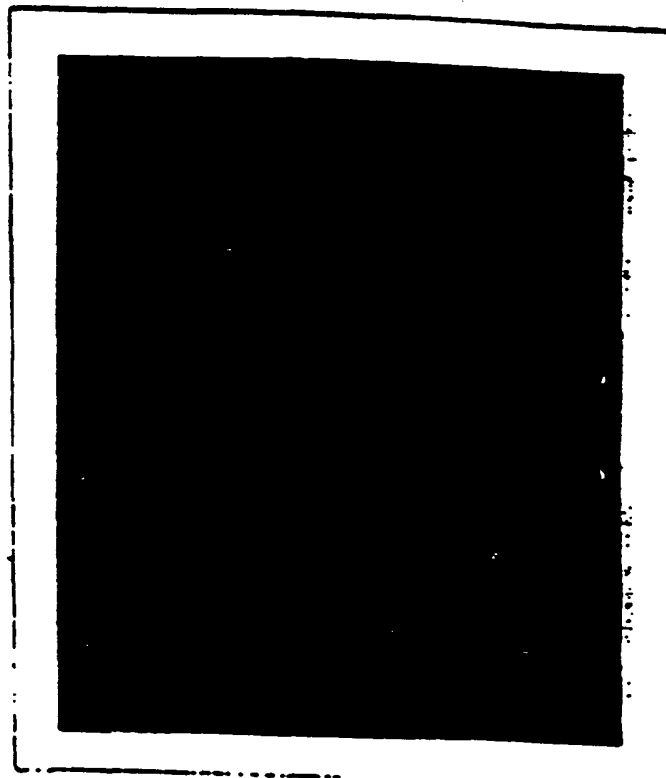
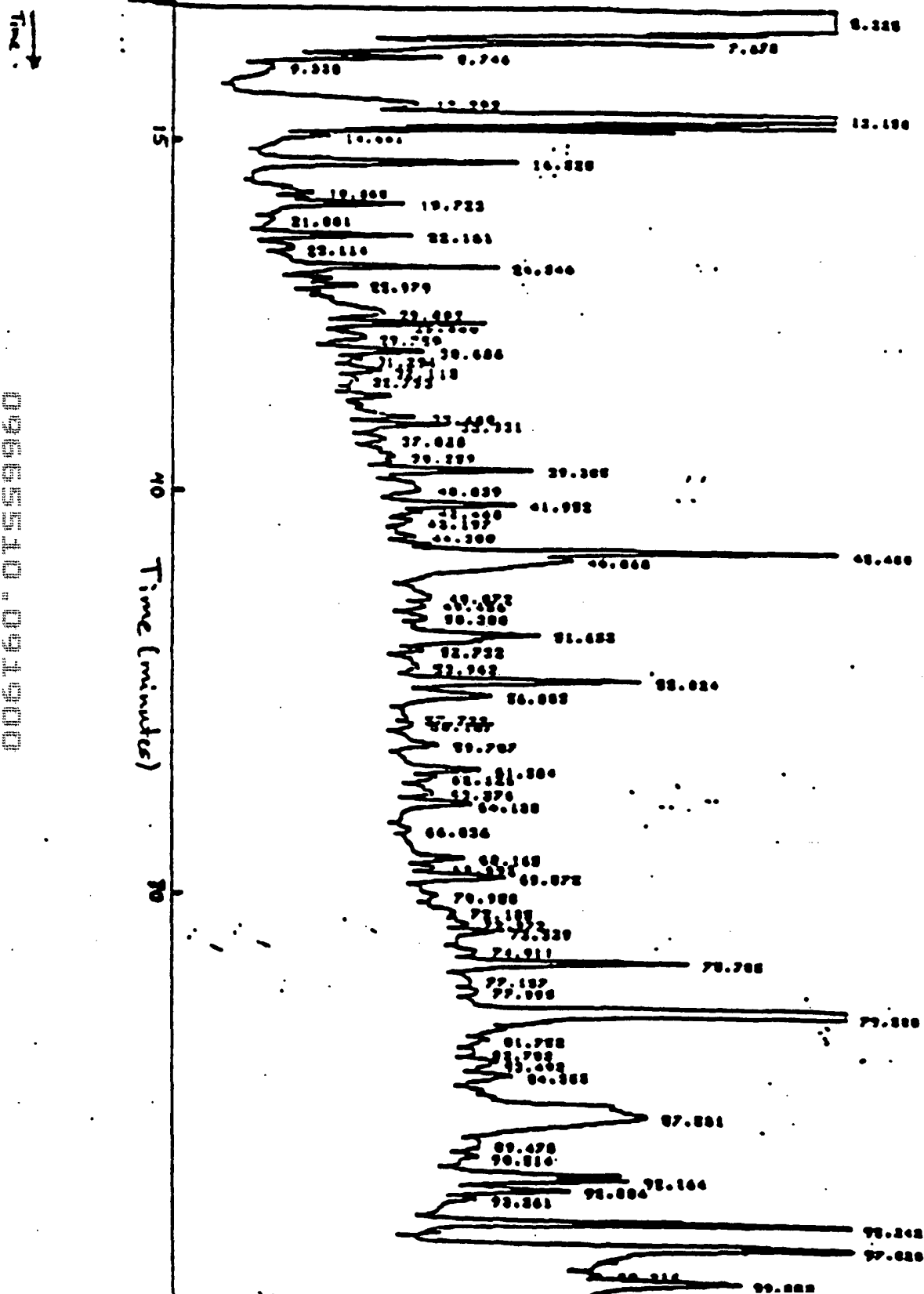


Figure 2

Peptides from HLA A3

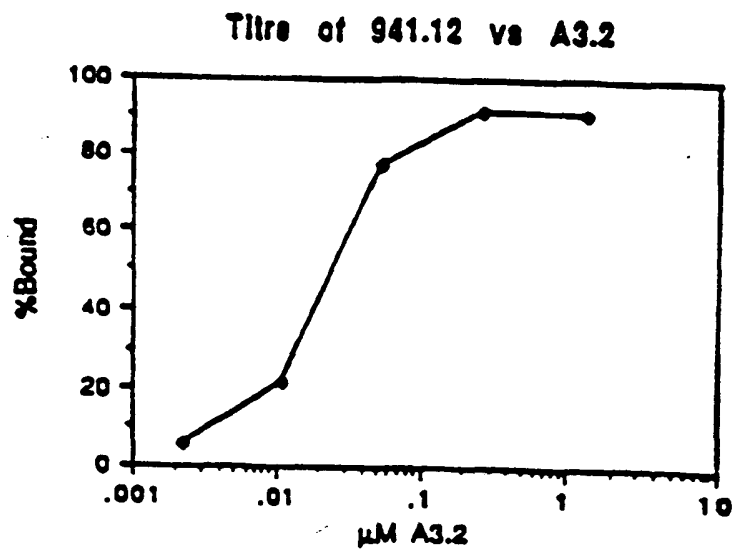
Wavelength	Absorbance
2.645	A214



RP-HPLC separation of HLA-A3 acid eluted peptides.

[illegible]

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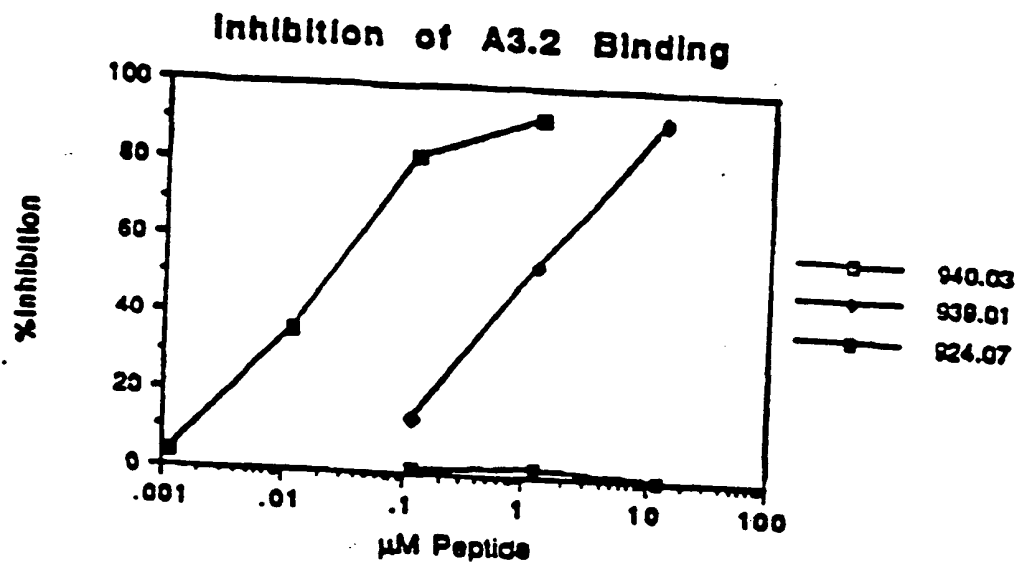


Figure 5

0066510-091900

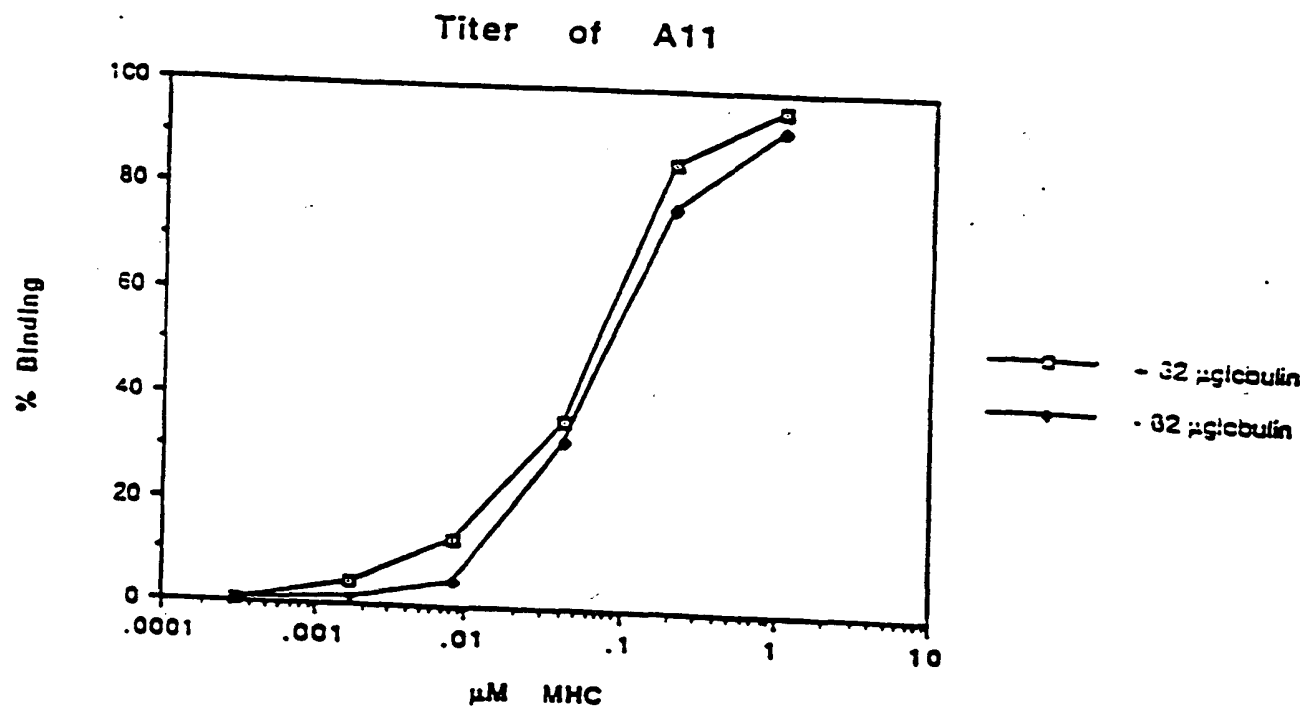


Figure 6

006760-0159 Inhibition

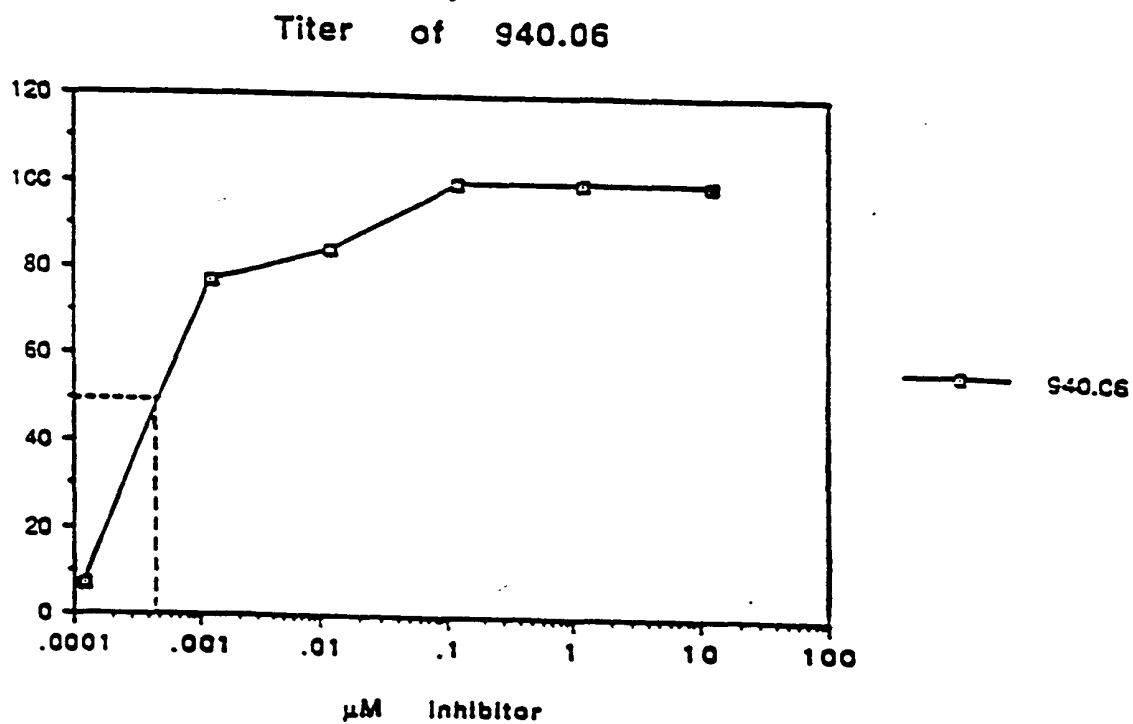


Figure 7

006160-07559960

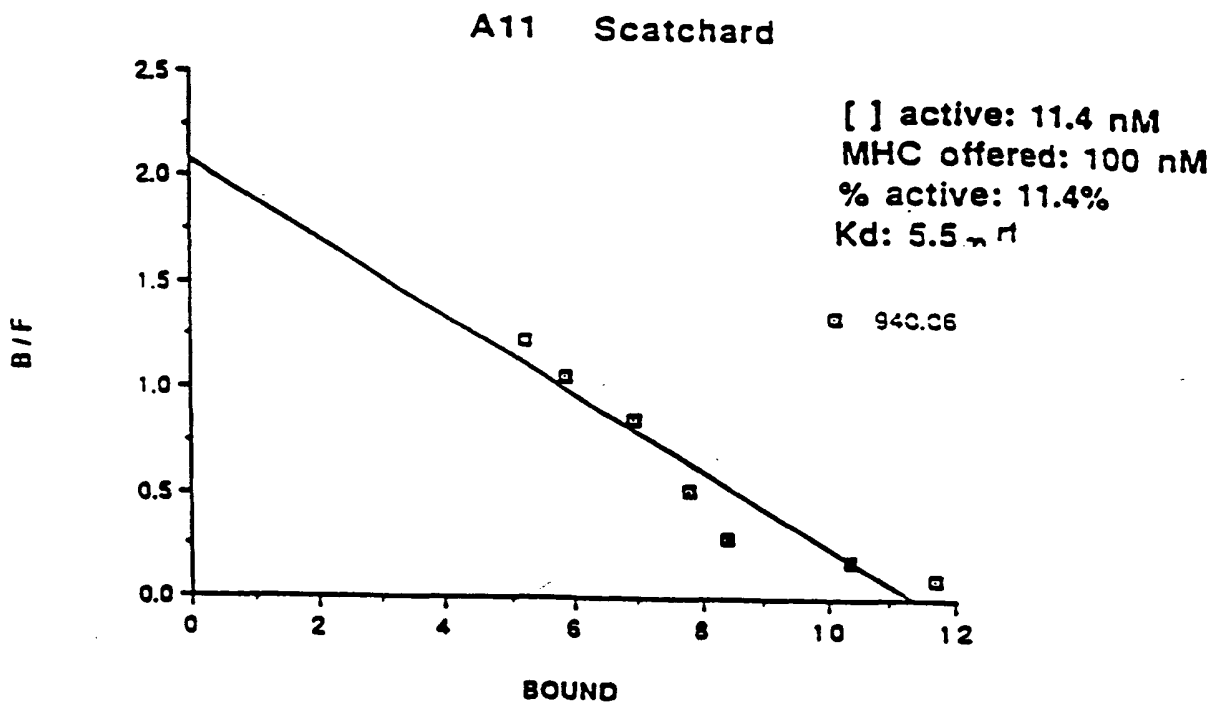


Figure 8

Titer of 944.02 against A1.

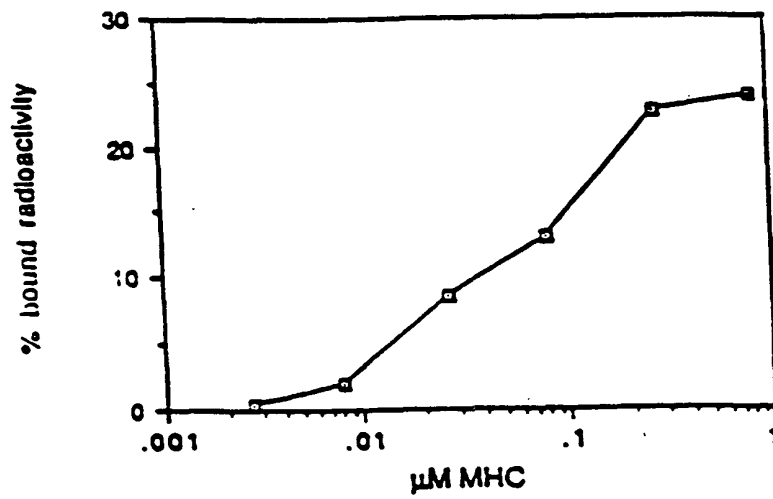


Figure 9

006760" 07559960

AI inhibition titration.

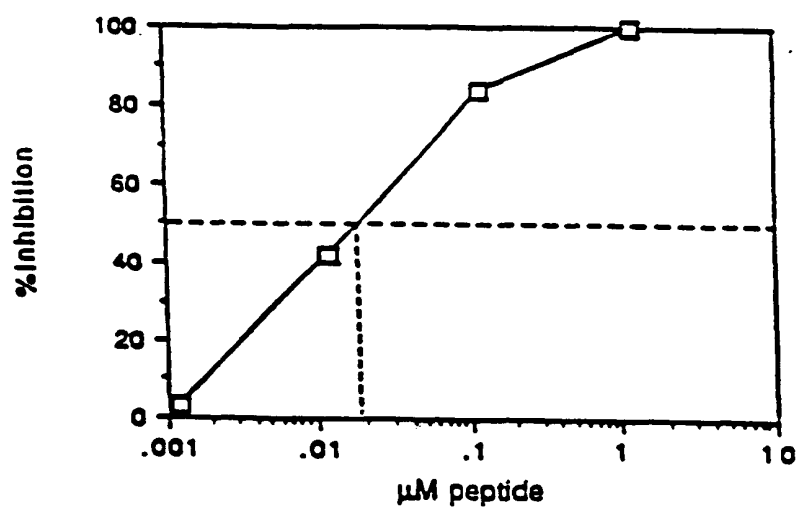


Figure 10

006510 055960

Scatchard for A1 vs. 944.02

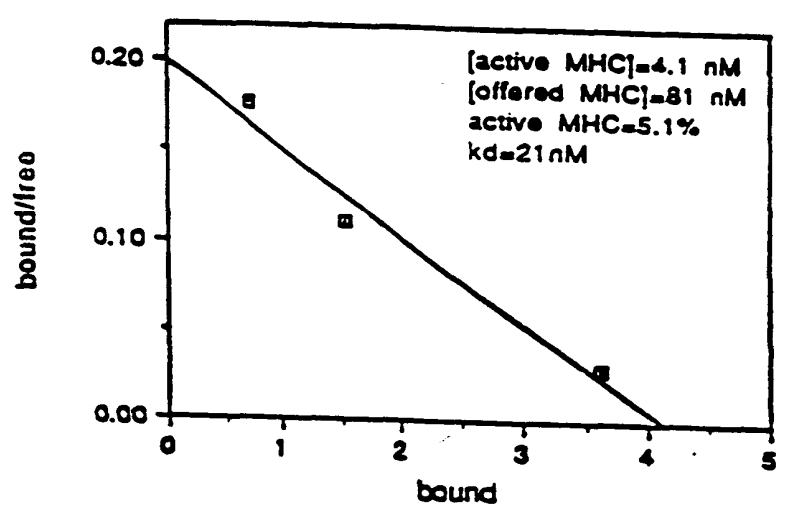


Figure 11

006T60-0T559960

A24 titration.

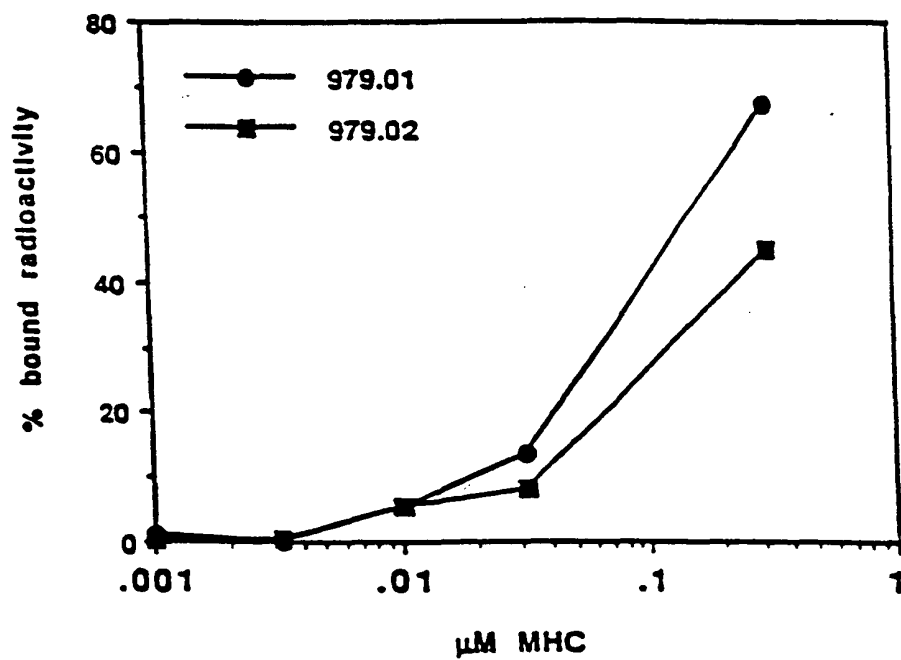


Figure 12

A24 Inhibition.

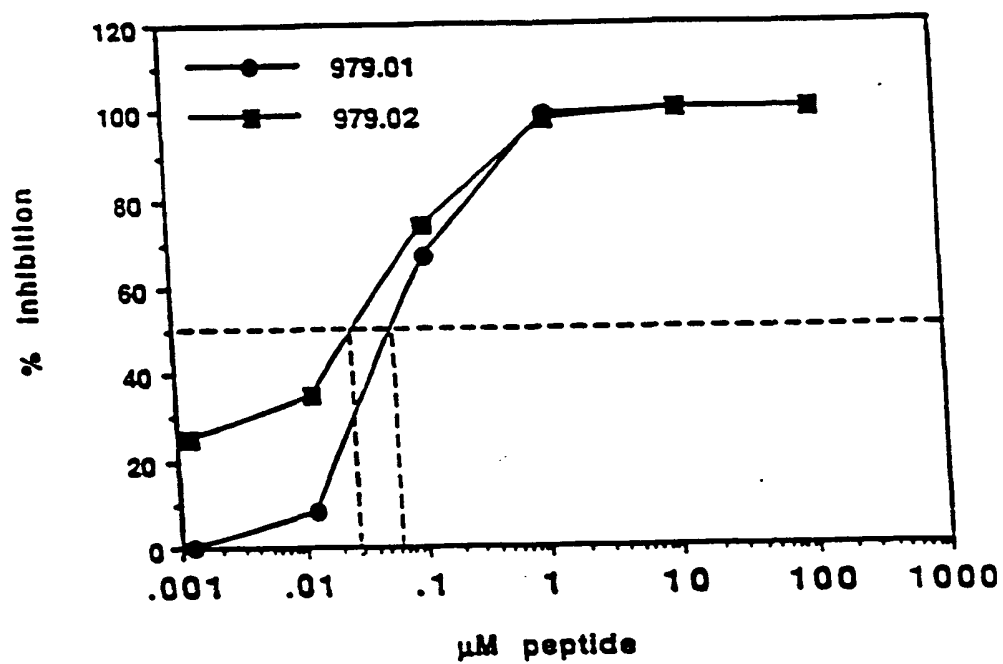


Figure 13

006T60-0759960

Figure 14A

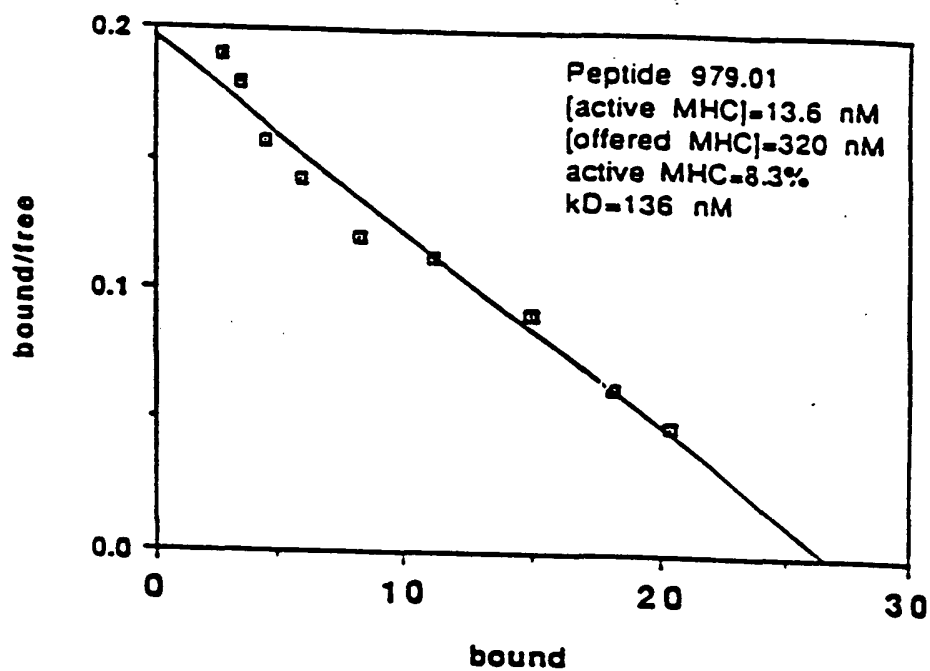


Figure 14B

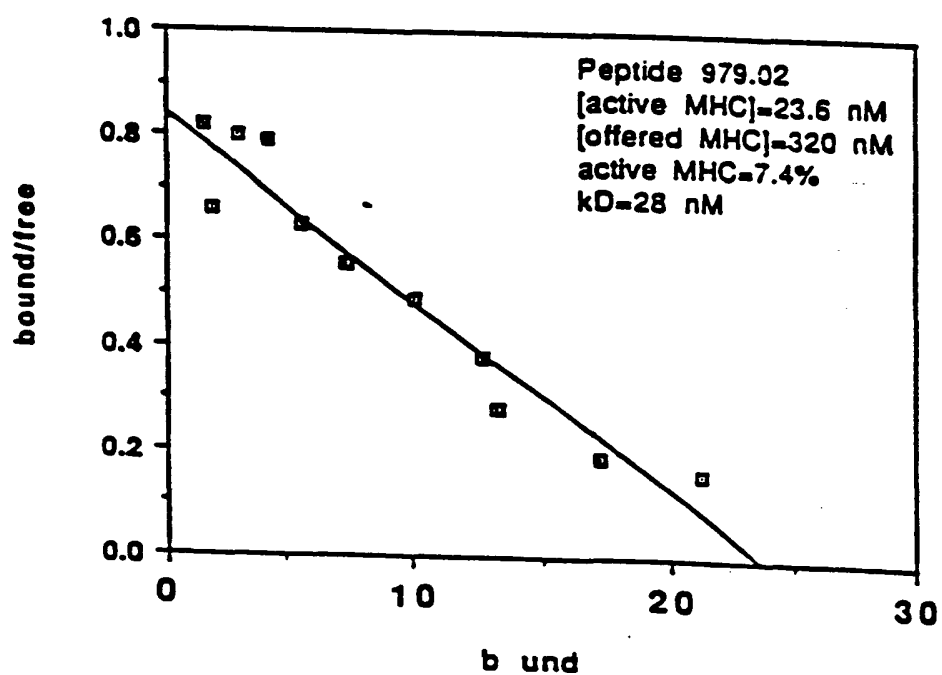


Figure 15

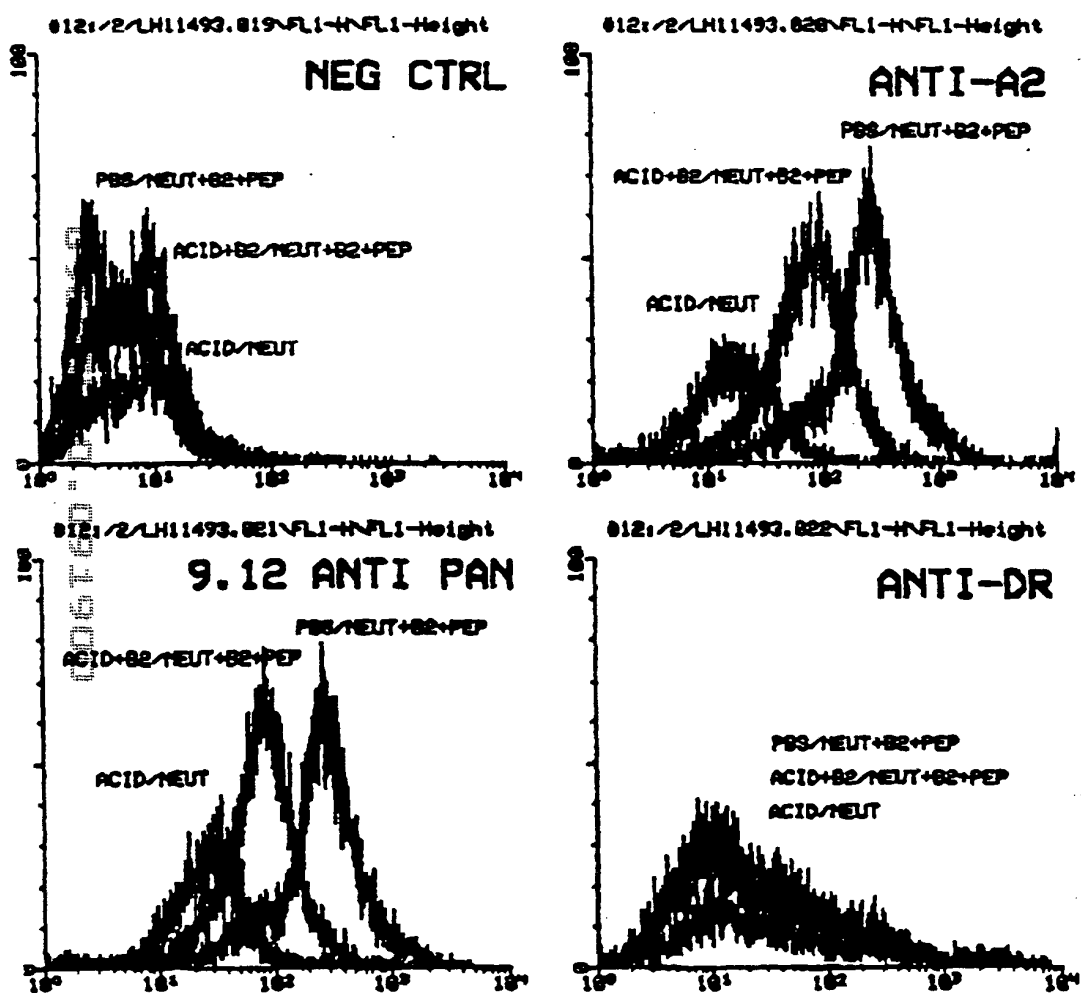


Figure 16

CTL INDUCTION
GC43 A2.1 CD4 DEPLETED RESPONDER
777.03 + 924.07 + 927.32 POOL

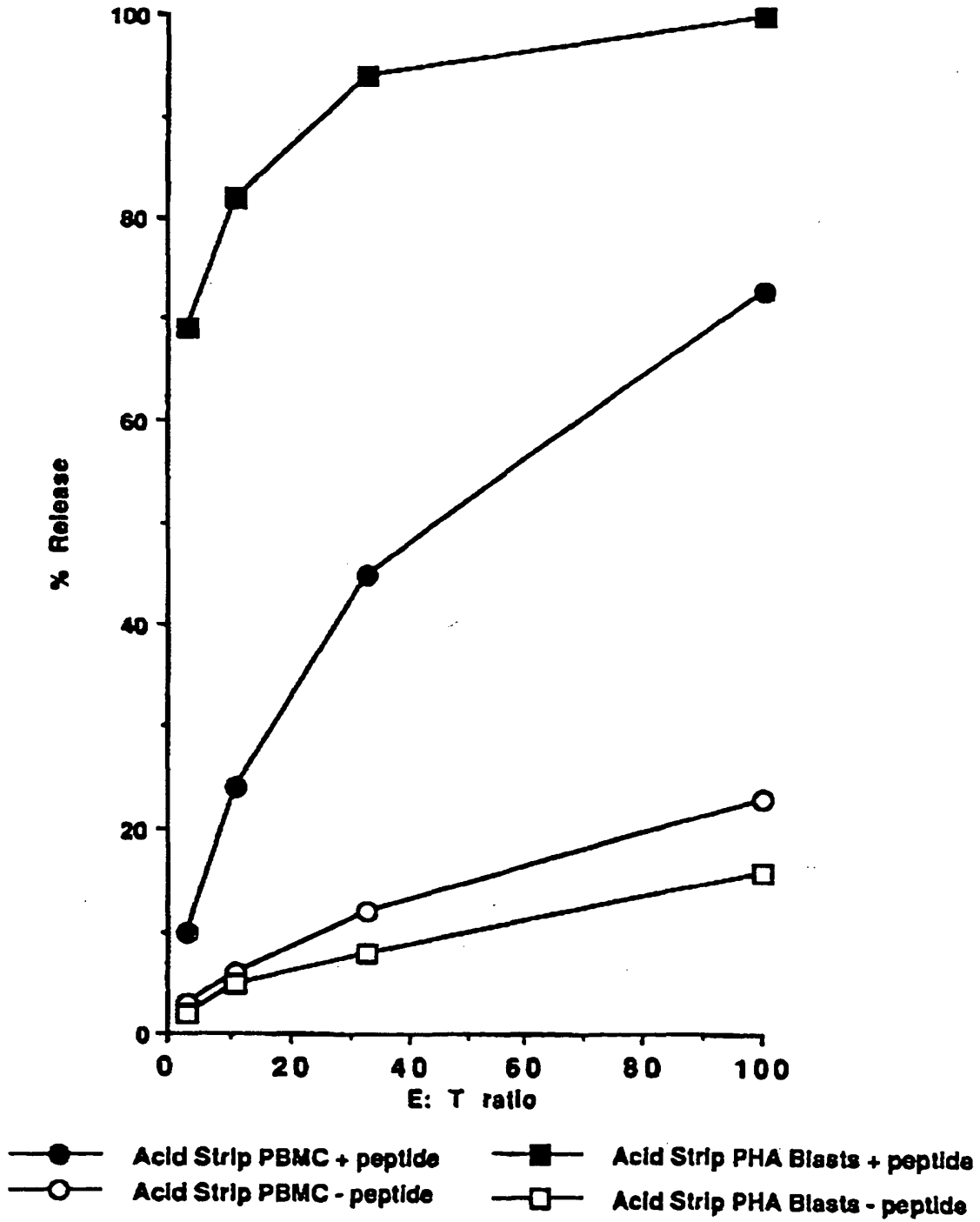
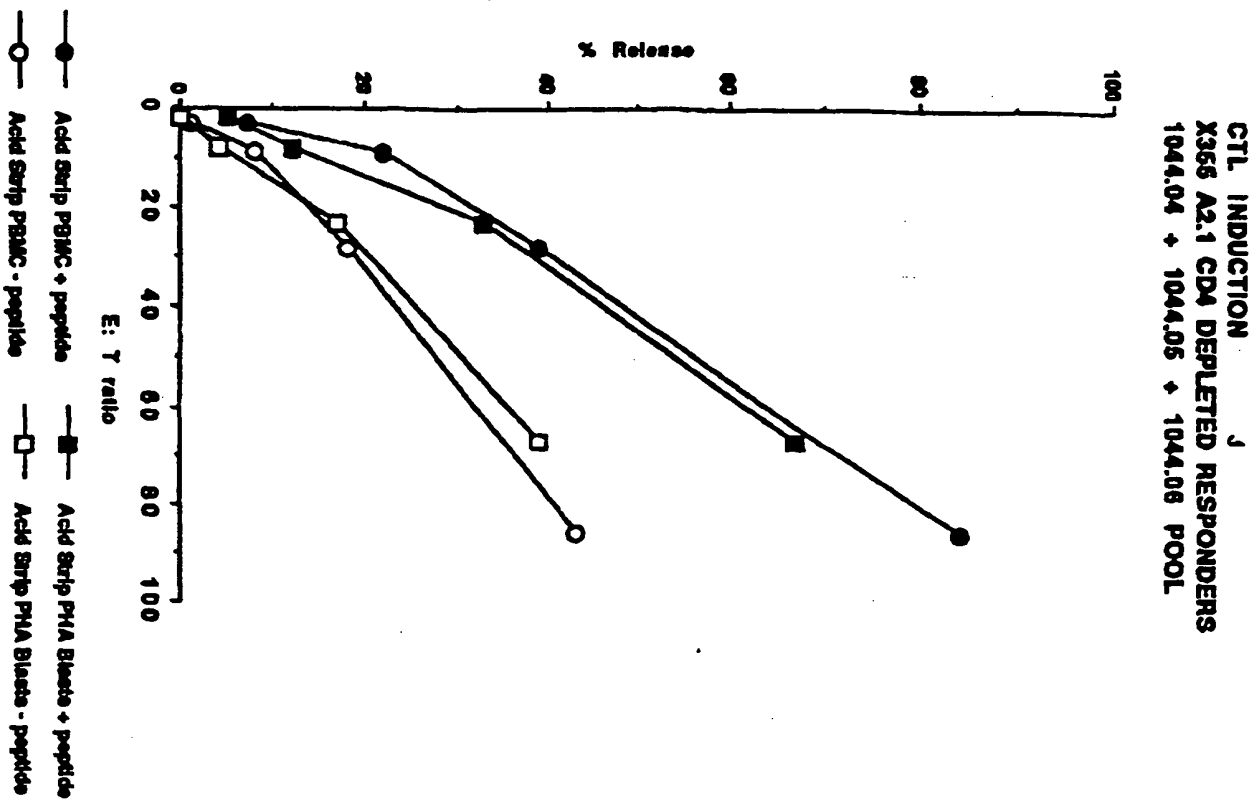
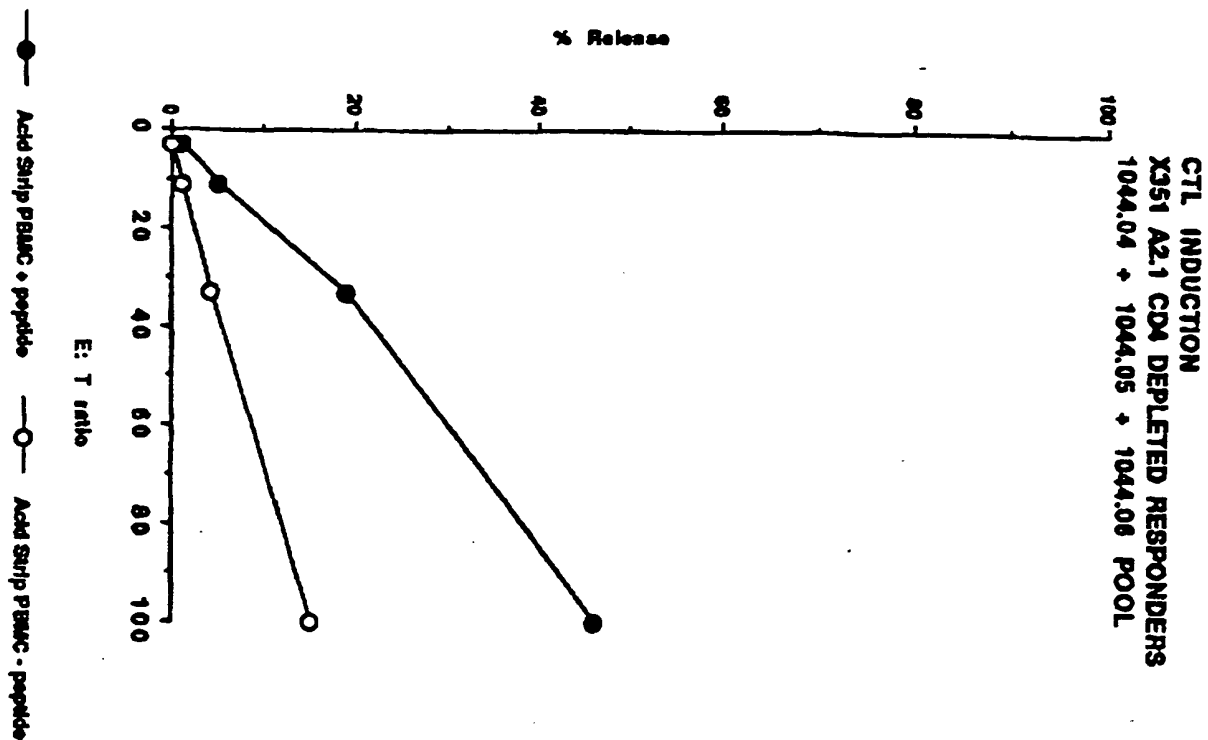


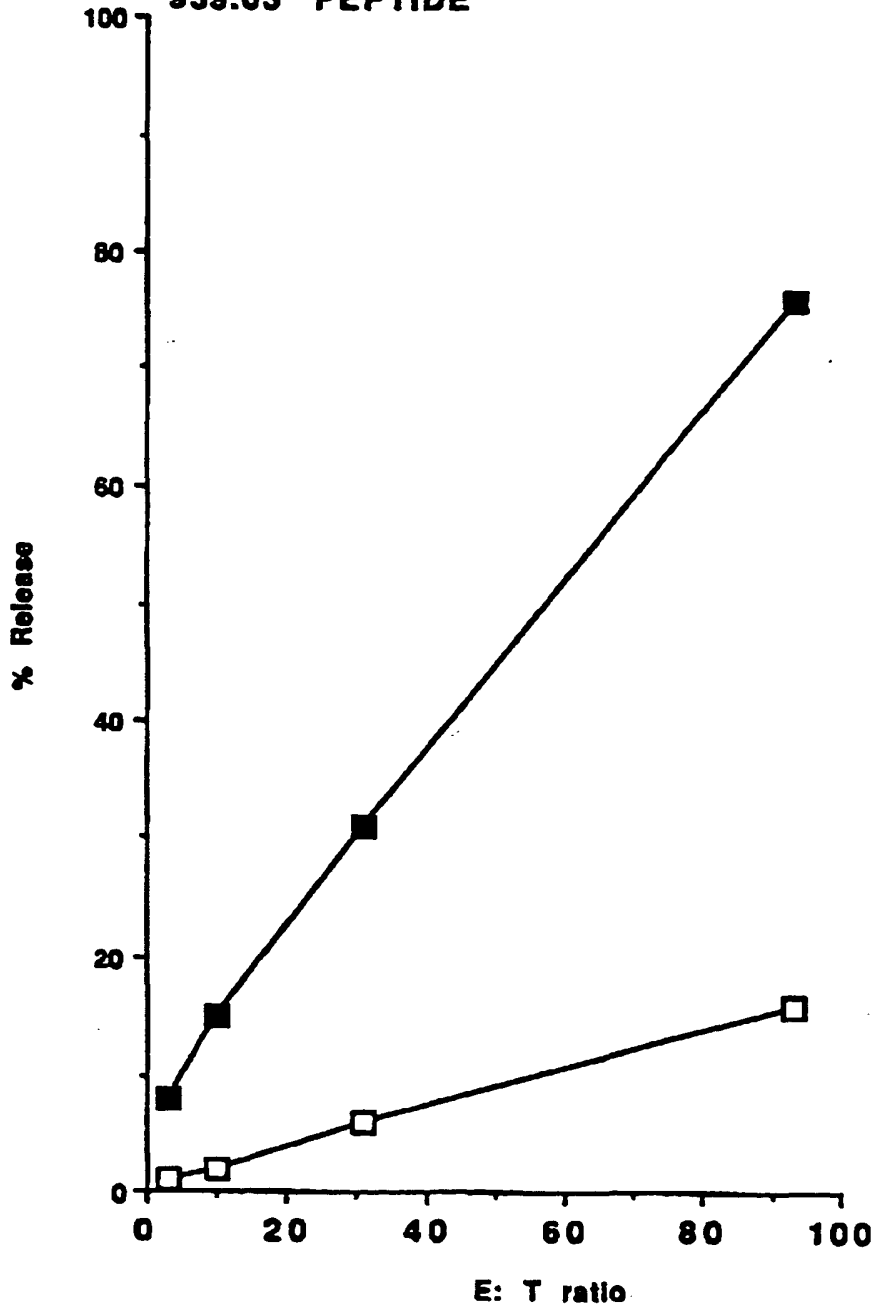
Figure 1 /



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Figure 18

CTL INDUCTION
GC49 A2.1 CD4 DEPLETED RESPONDERS
939.03 PEPTIDE



—■— Acid Strip PHA Blasts + peptide —□— Acid Strip PHA Blasts - peptide

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Figure 19

MAGE1 Peptide Specific, HLA-A1-Restricted CTL Can Kill Melanoma Cells Expressing the Endogenous Antigen

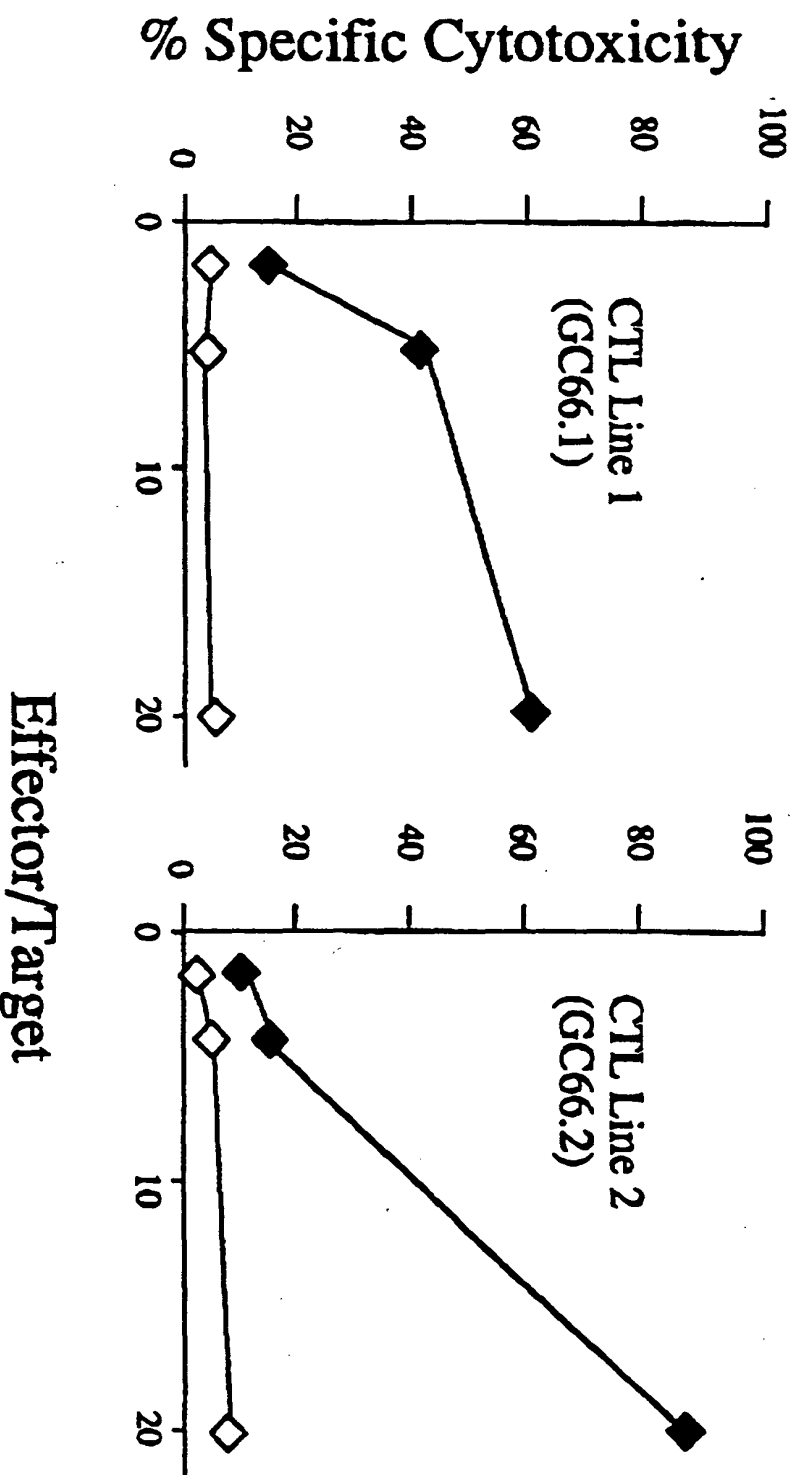
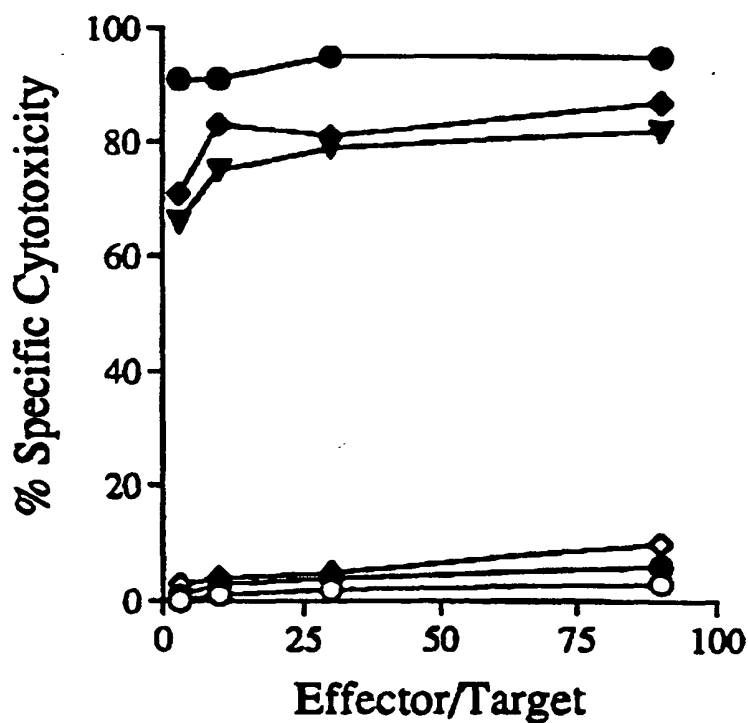


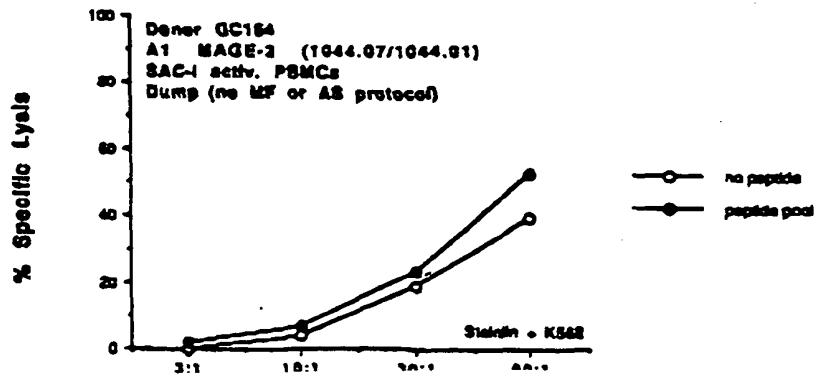
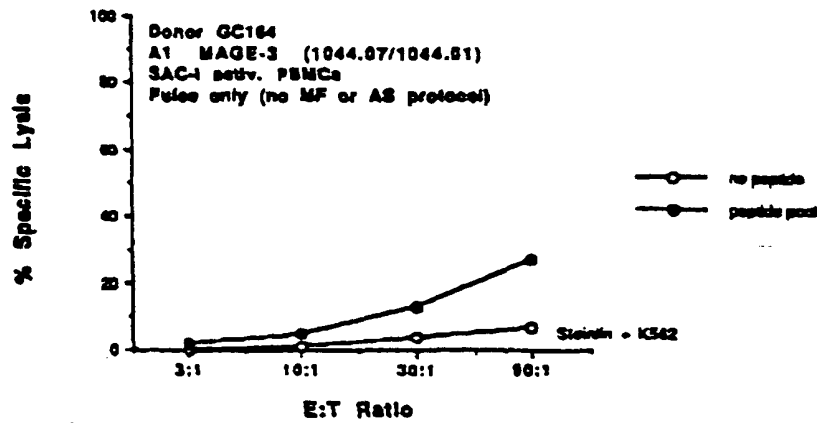
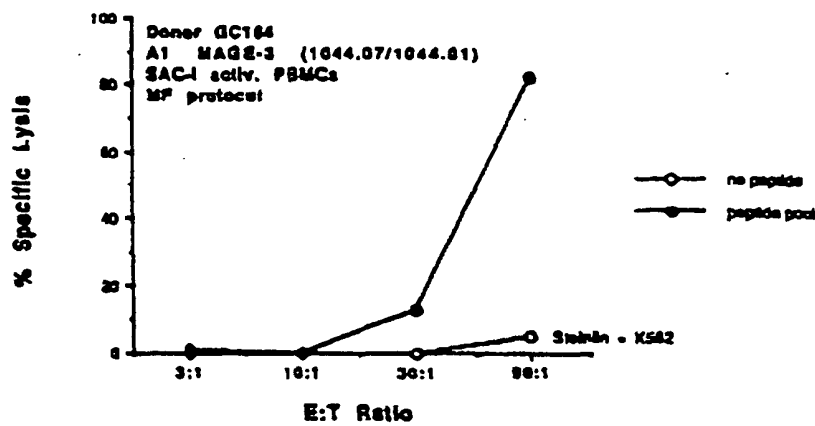
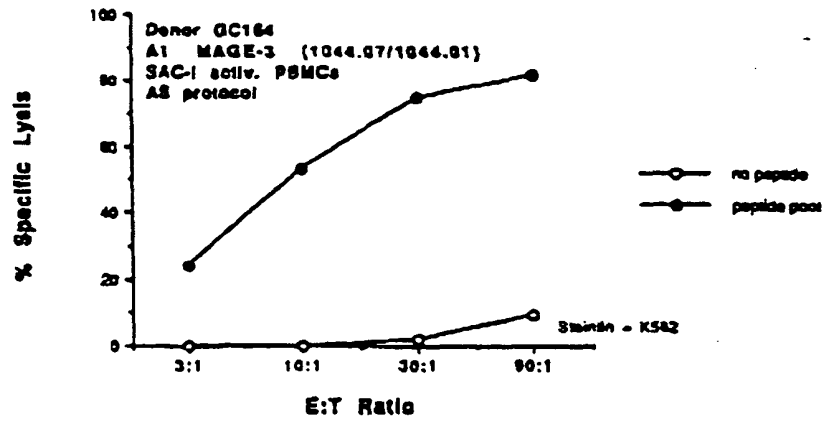
Figure 20

Cytotoxic Activity of an HLA-A1-Restricted CTL Line Specific for a MAGE3 Peptide (1044.07)



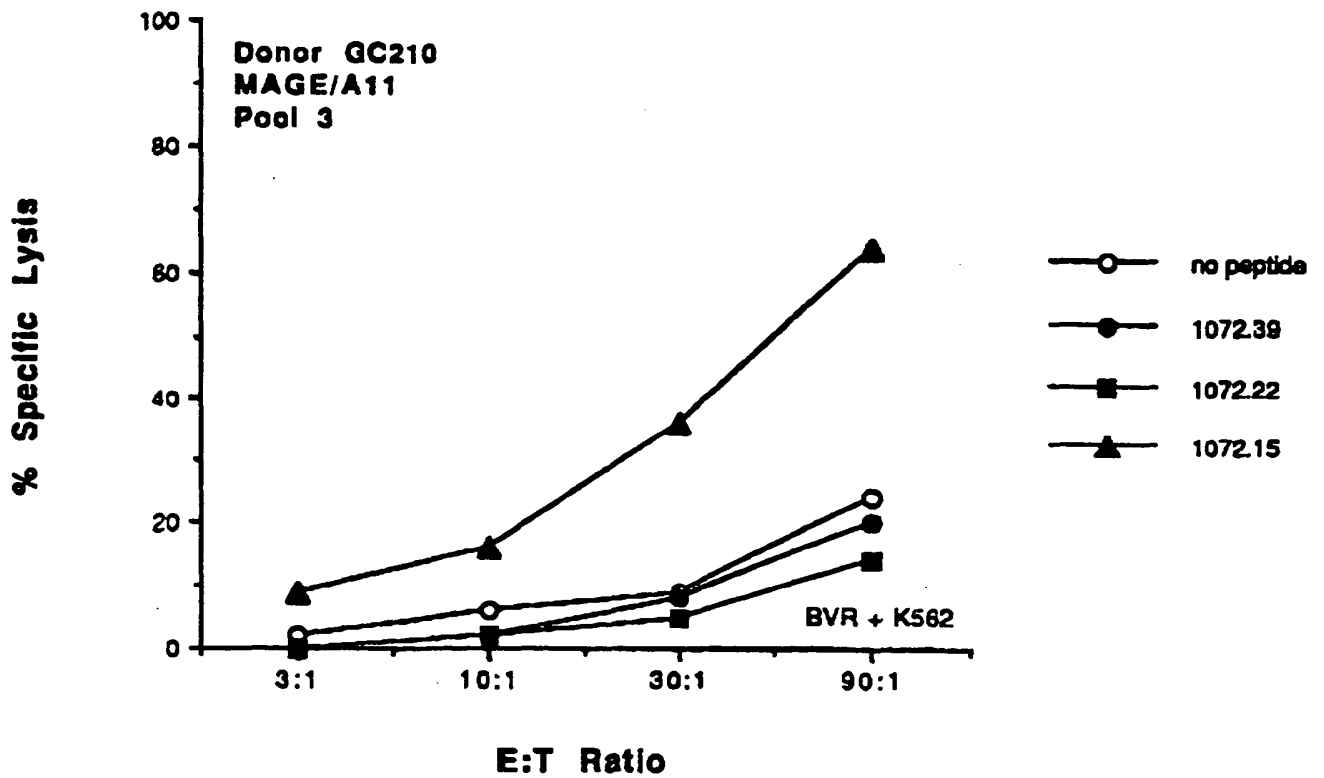
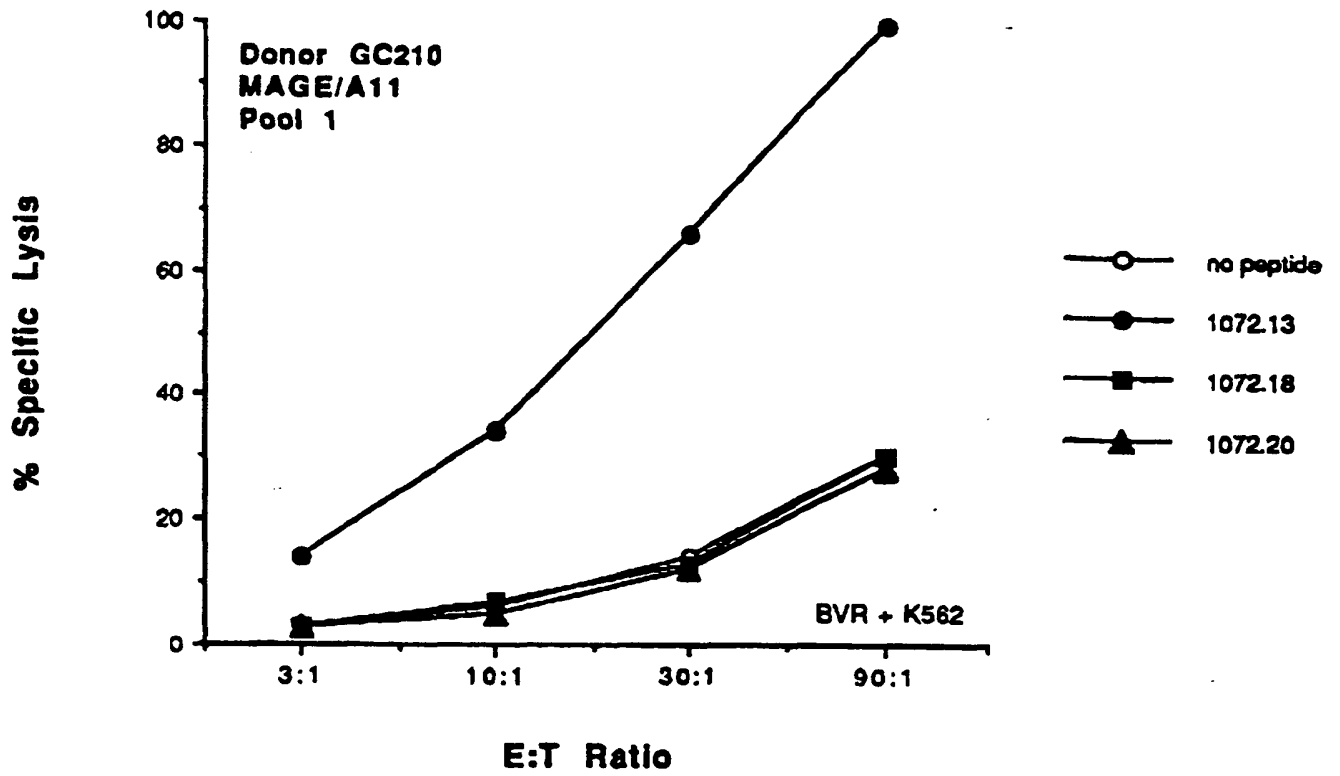
- | | |
|------------------------|------------------------|
| —○— Steinlin - Pep | —◇— mel 888 (MAGE-3 -) |
| —●— Steinlin + 1044.01 | —▼— mel 397 (MAGE-3 +) |
| —●— Steinlin + 1044.07 | —◆— mel 938 (MAGE-3 +) |

Induction Using Different Methods to Load Peptides onto SAC-I C IIs.



006160-0159360

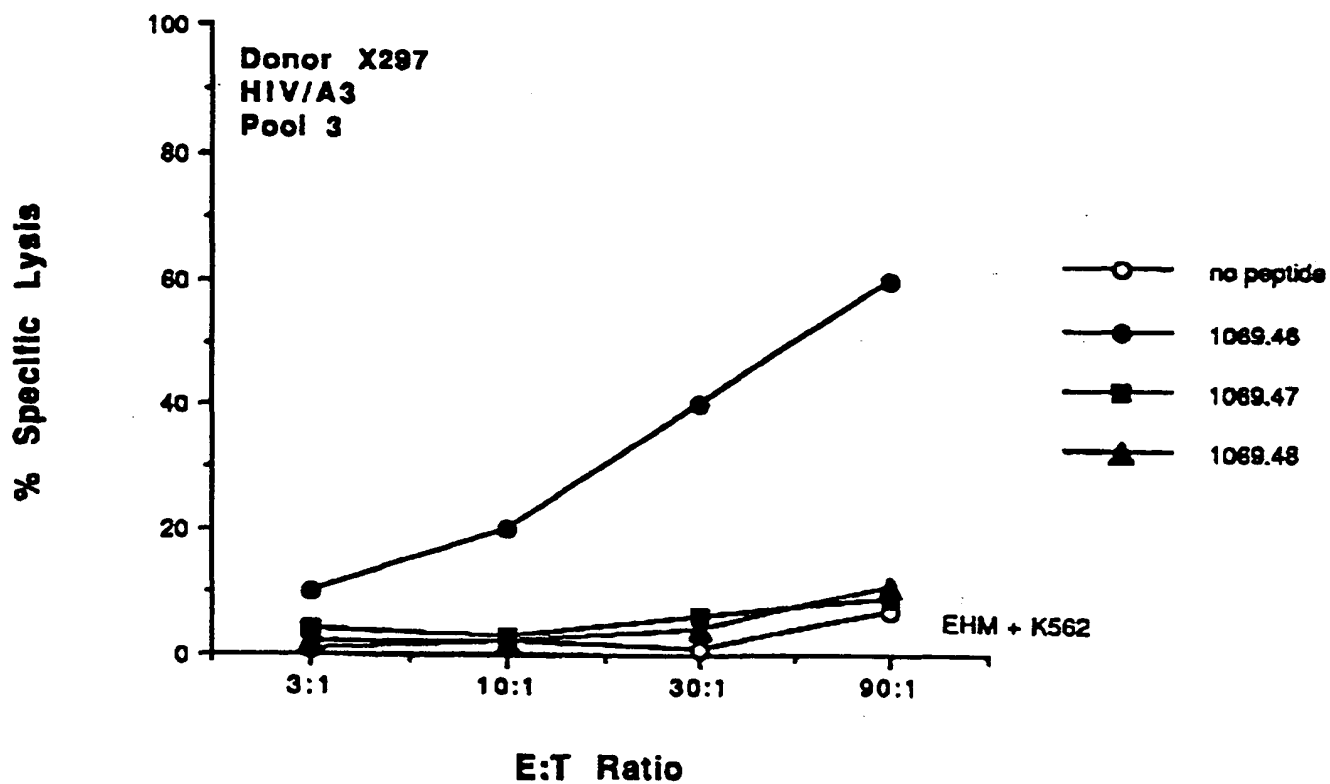
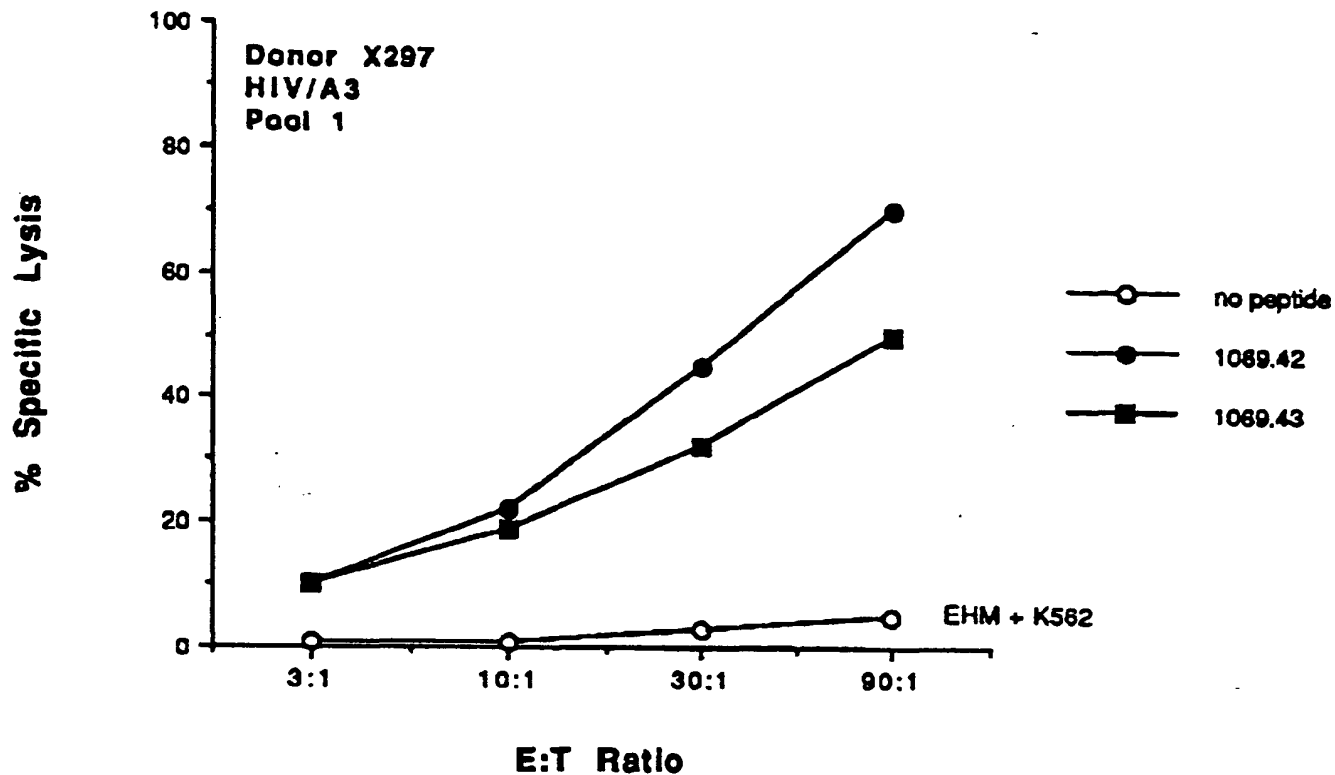
Figur 22
Peptide Screening:
MAGE/A11



006T60-0T559960

Figure 23

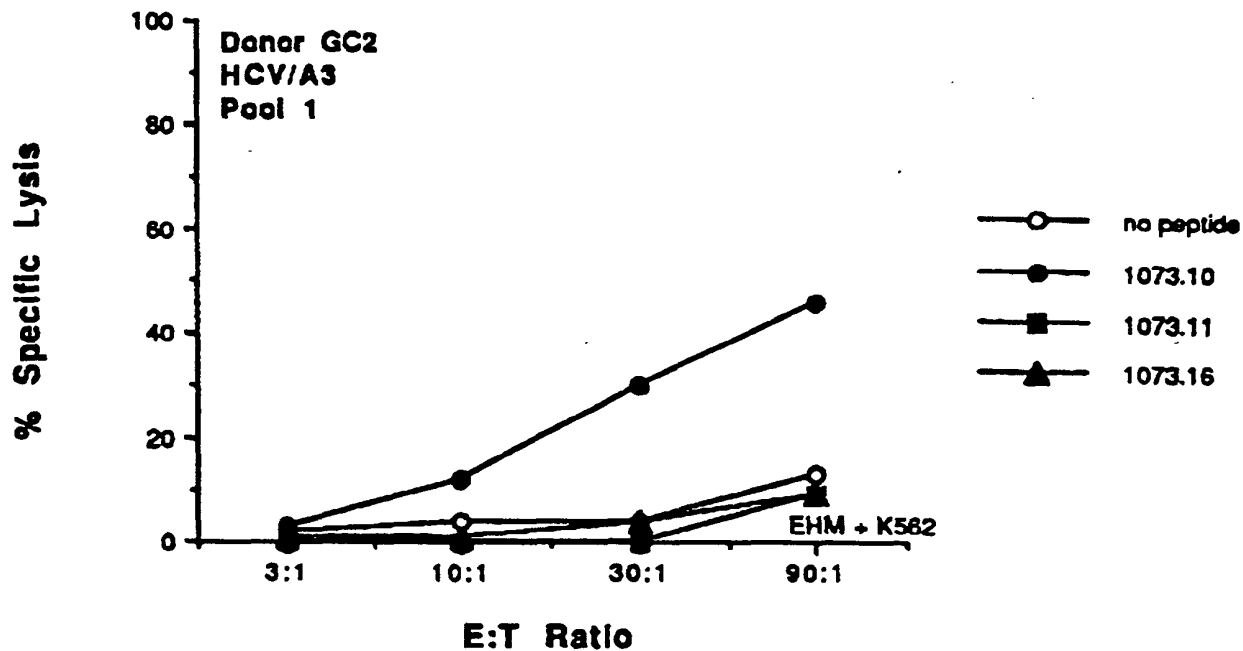
**Peptide Screening:
HIV/A3**



006160-01559960

Figure 24

Peptide Screening:
HCV/A3



006T60-07559360

Figure 25

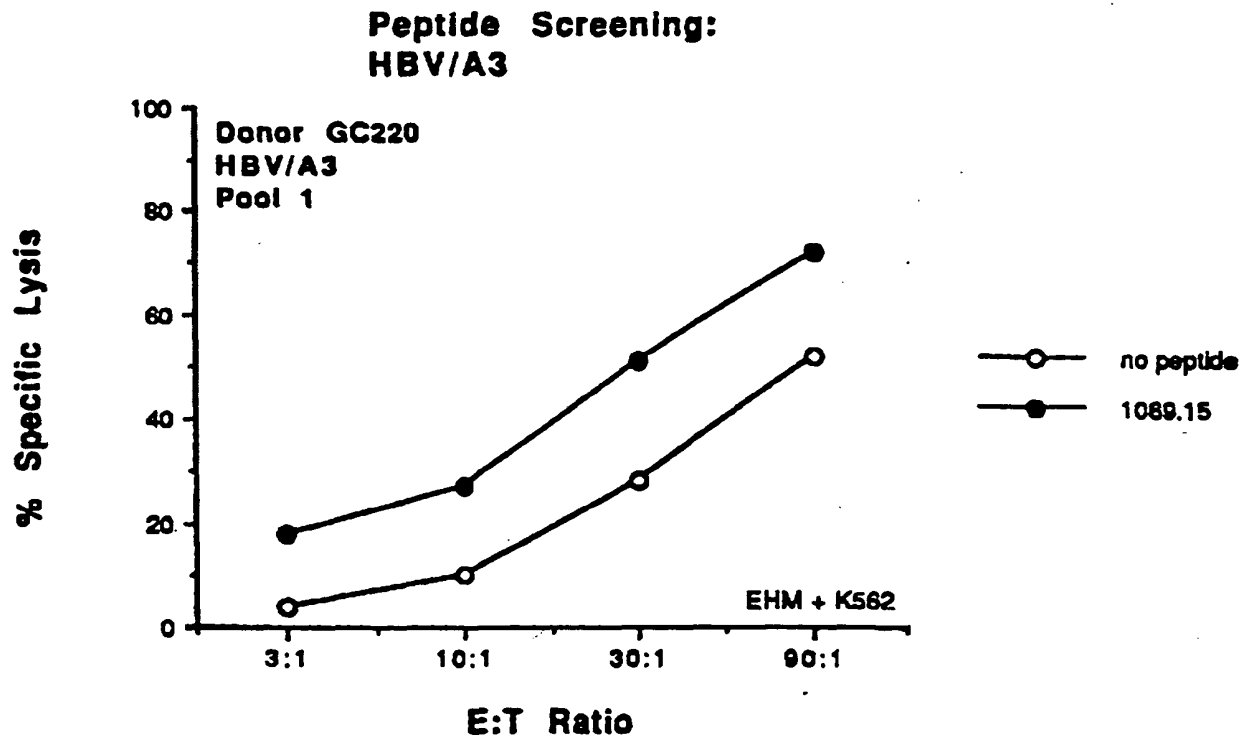


Figure 15

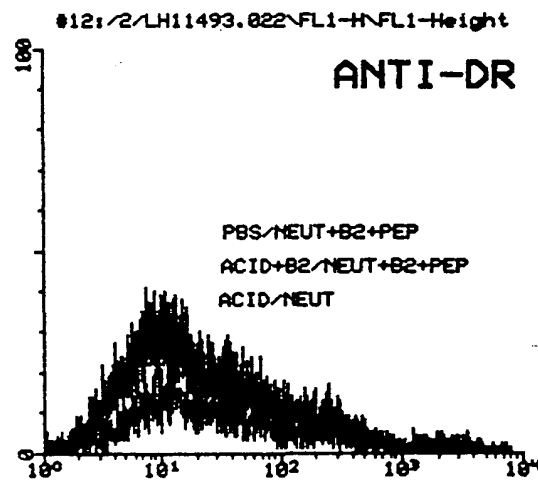
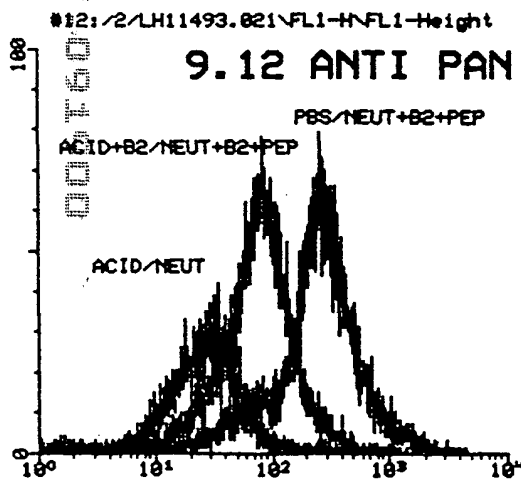
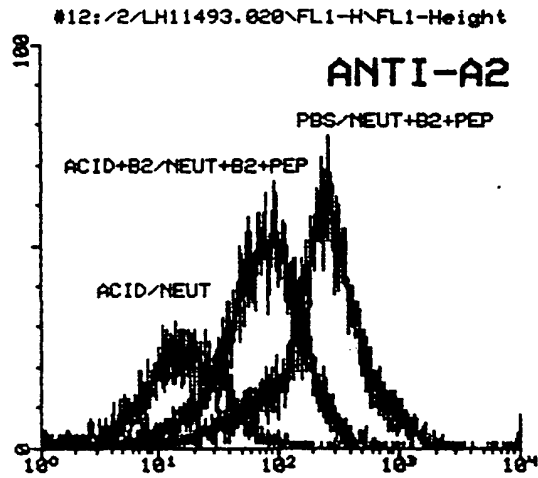
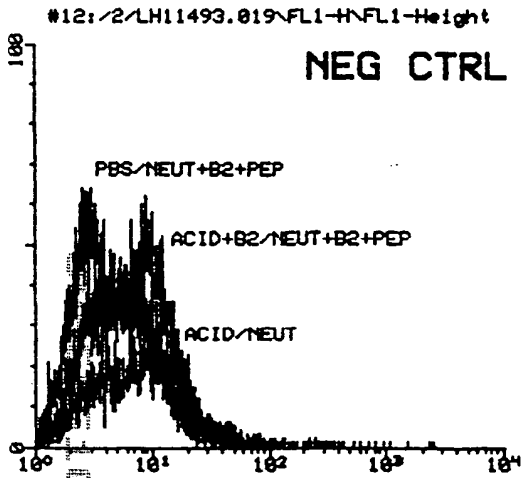


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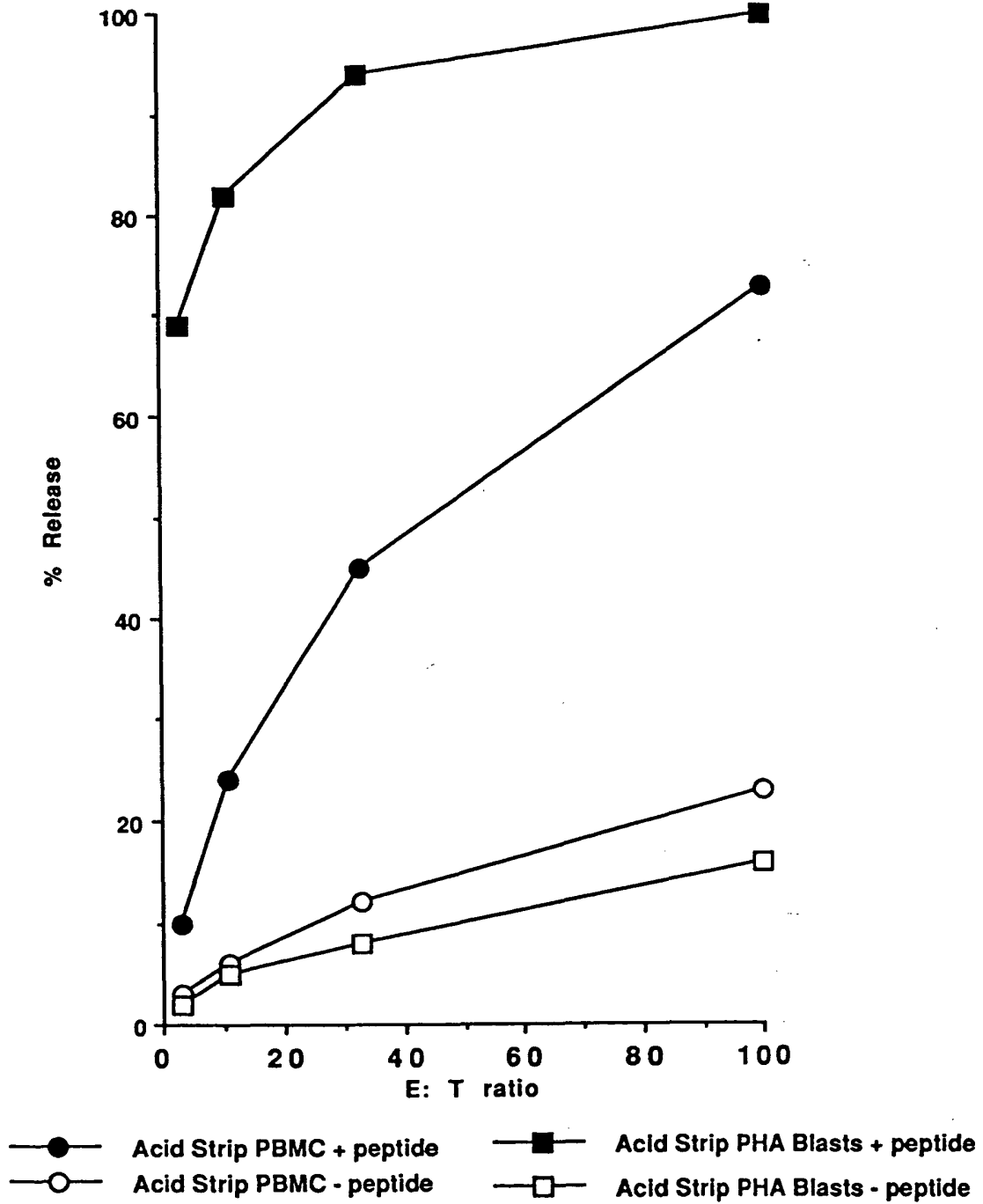
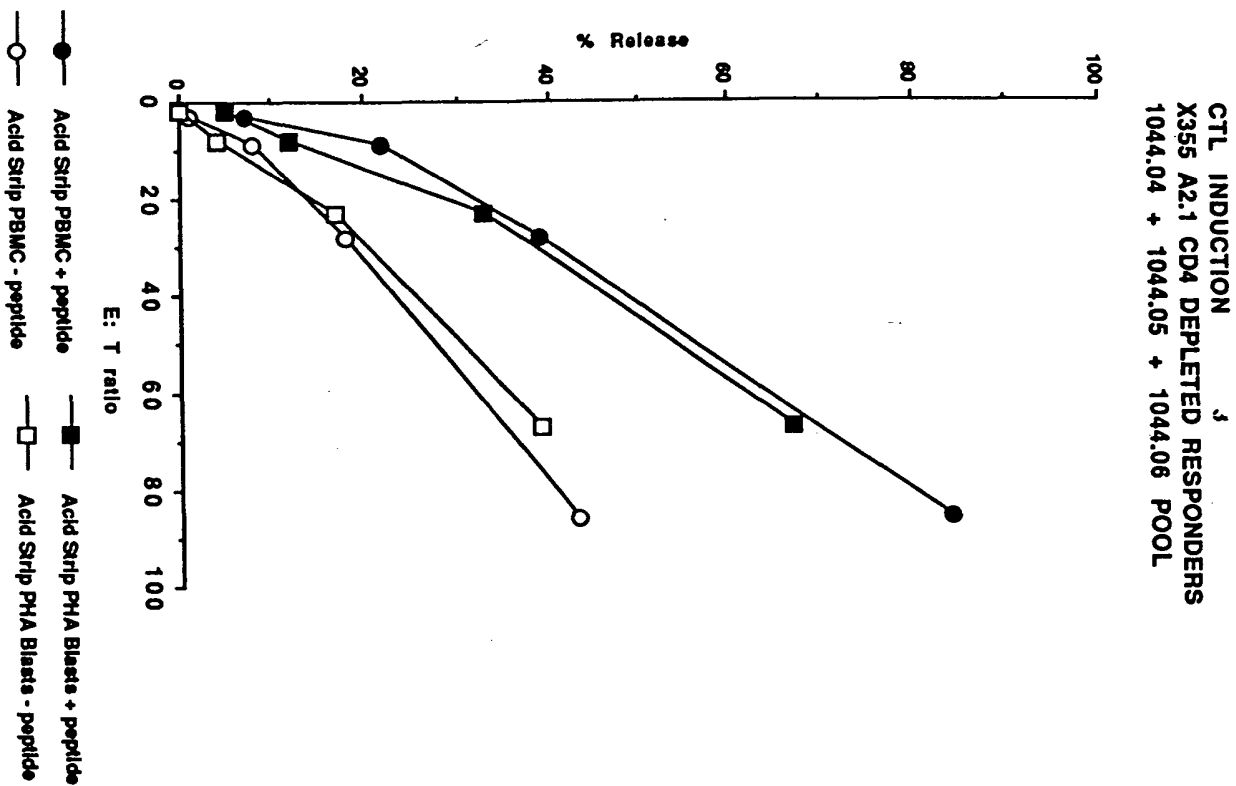
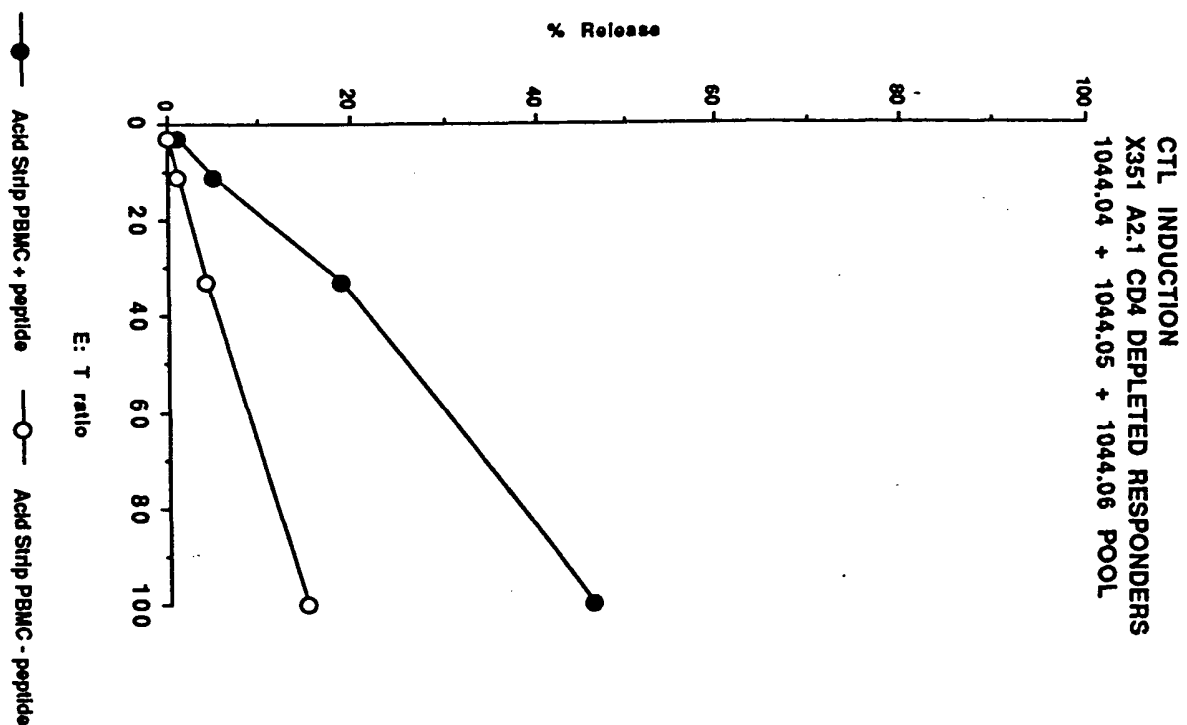


Figure 17



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Figure 18

CTL INDUCTION
GC49 A2.1 CD4 DEPLETED RESPONDERS
939.03 PEPTIDE

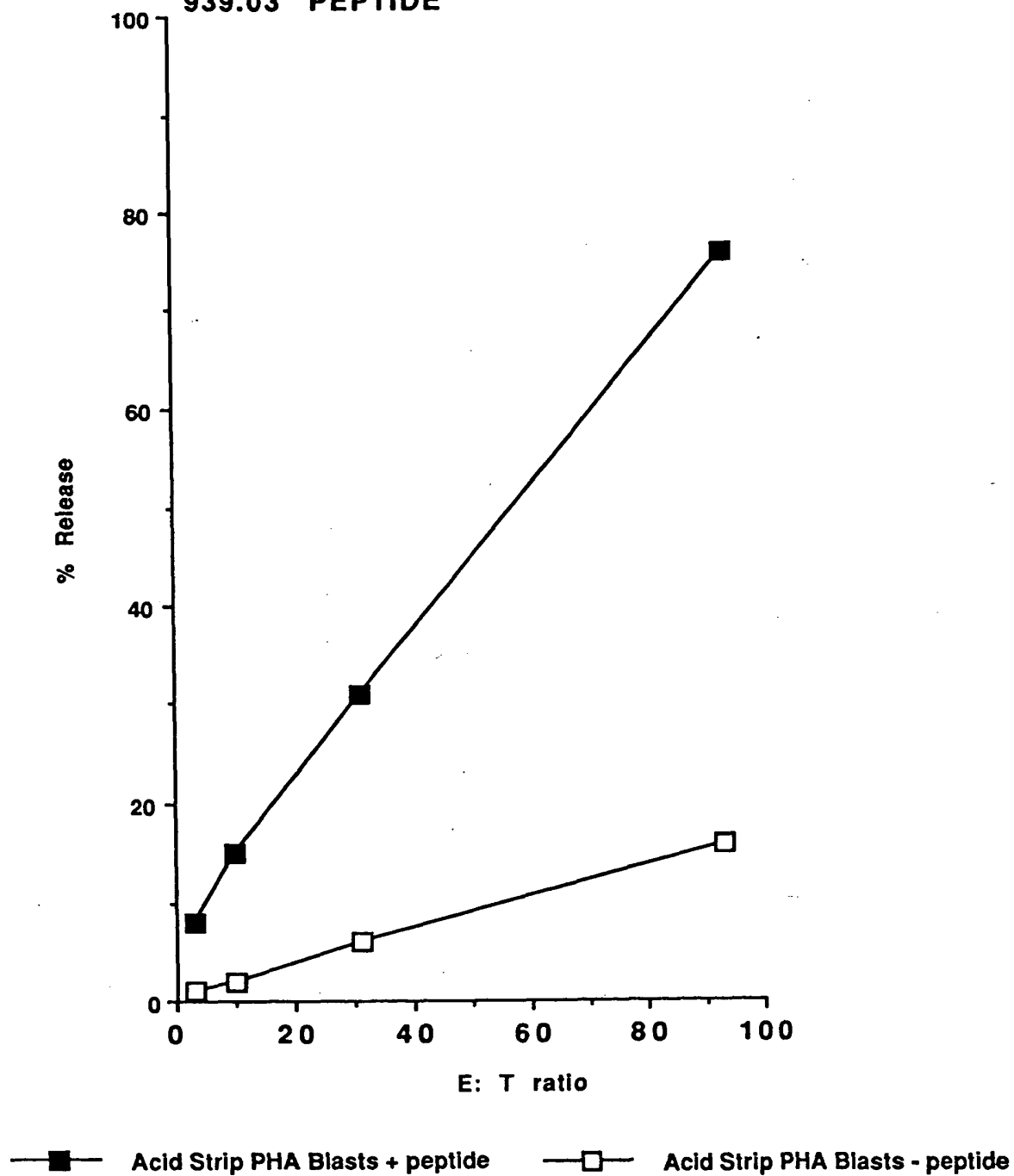


Figure 19

MAGE1 Peptide Specific, HLA-A1-Restricted CTL Can Kill Melanoma Cells Expressing the Endogenous Antigen

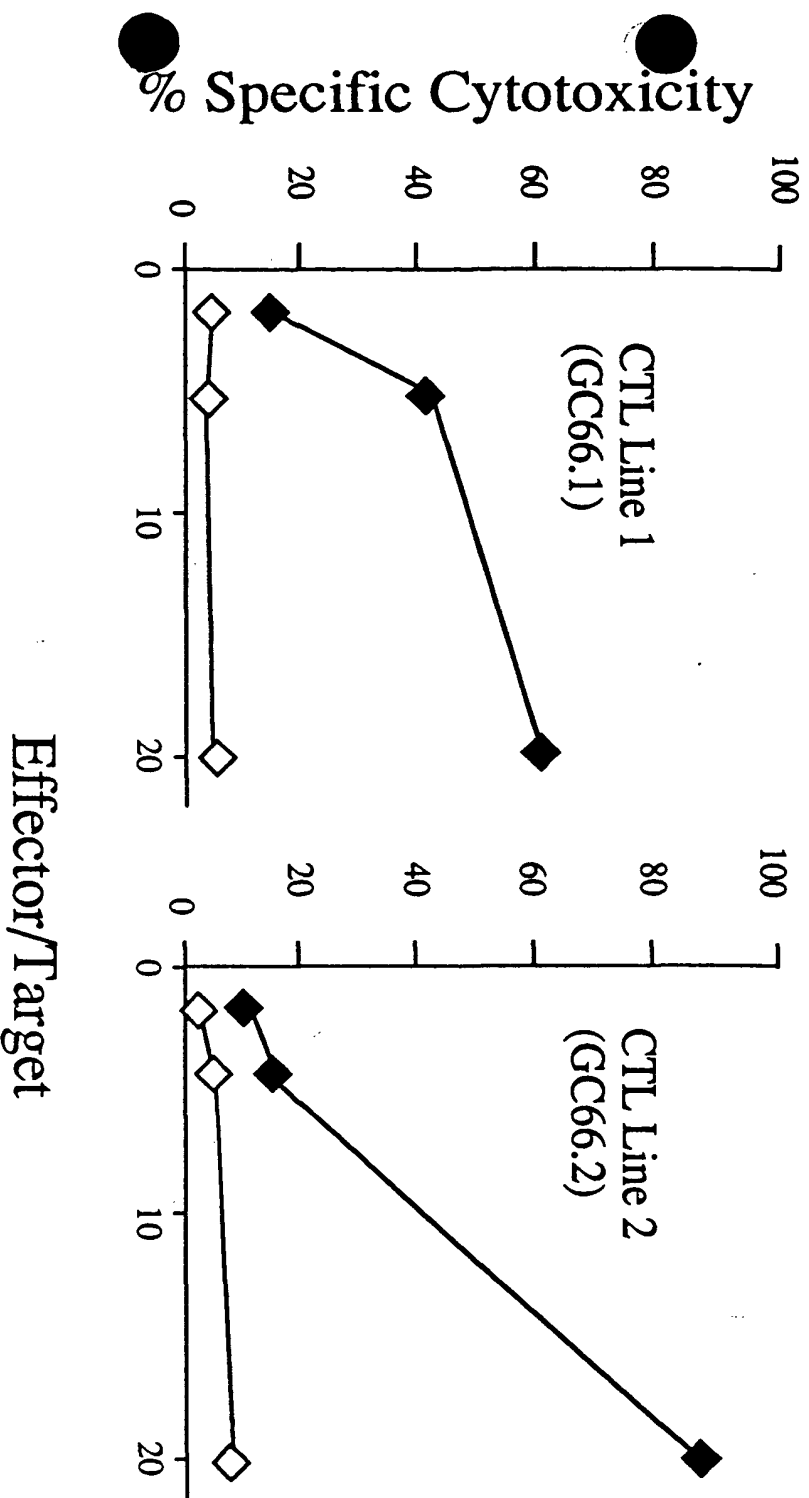
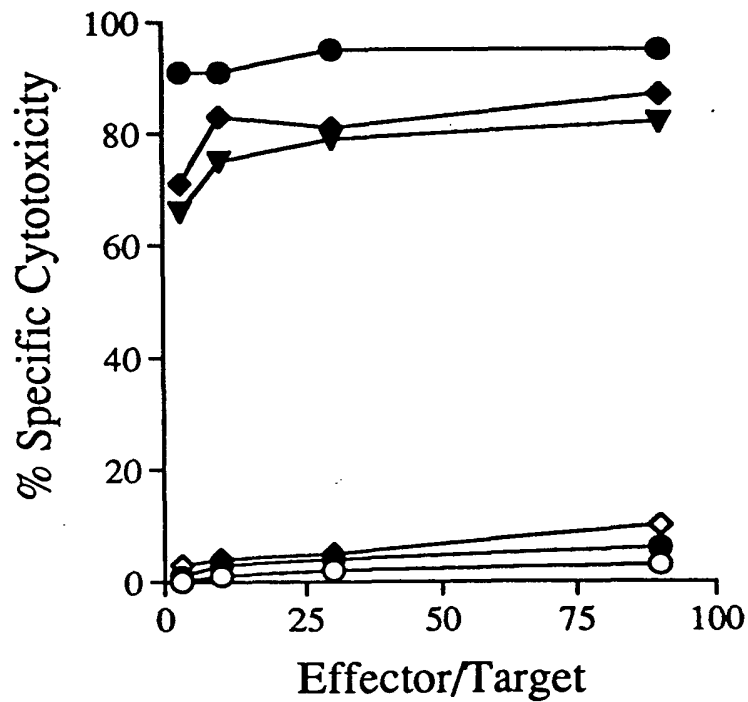


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- | | | | |
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Figure 21
Induction Using Different Methods
to Load P peptides onto SAC-I cells.

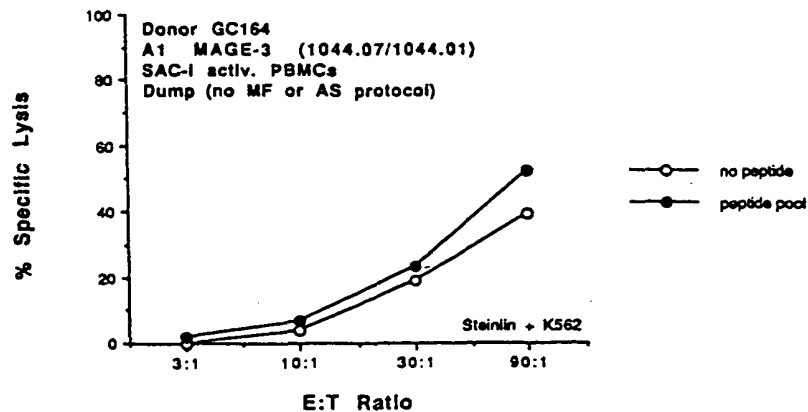
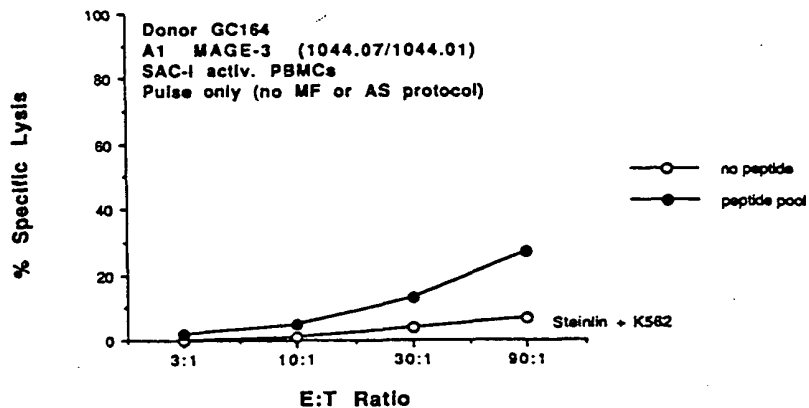
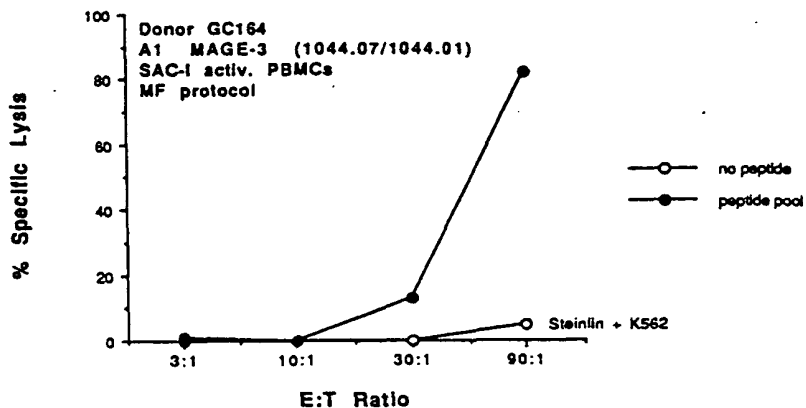
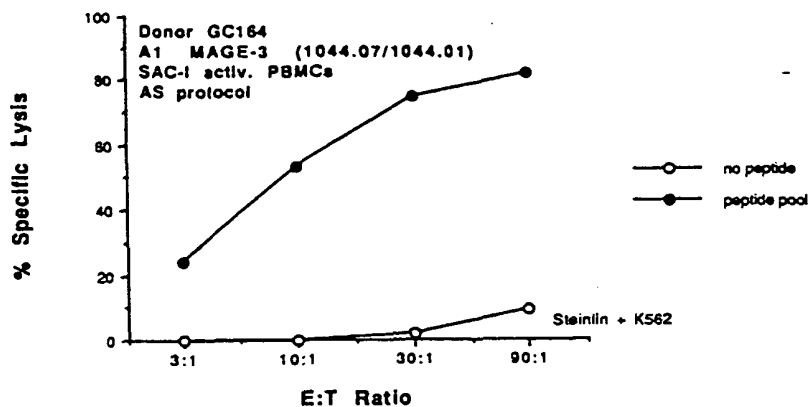


Figure 22
 Peptide Screening:
 MAGE/A11

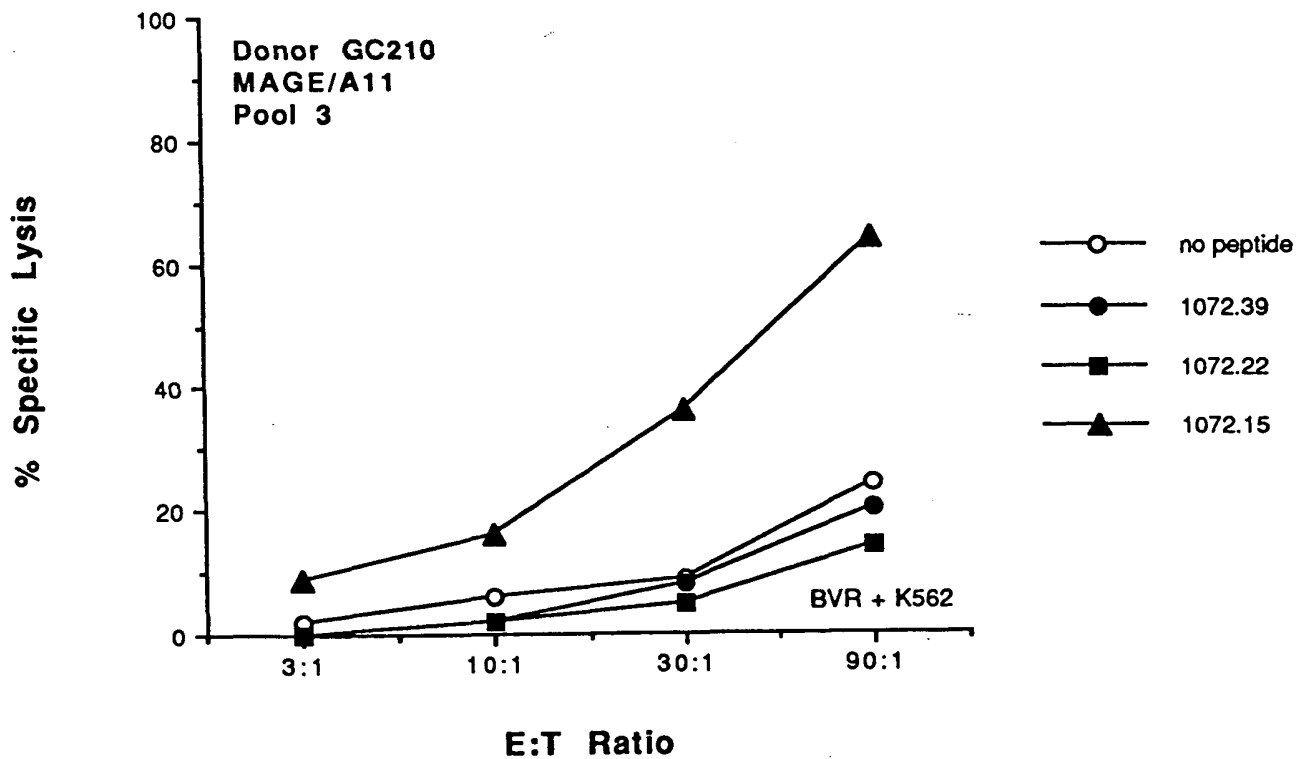
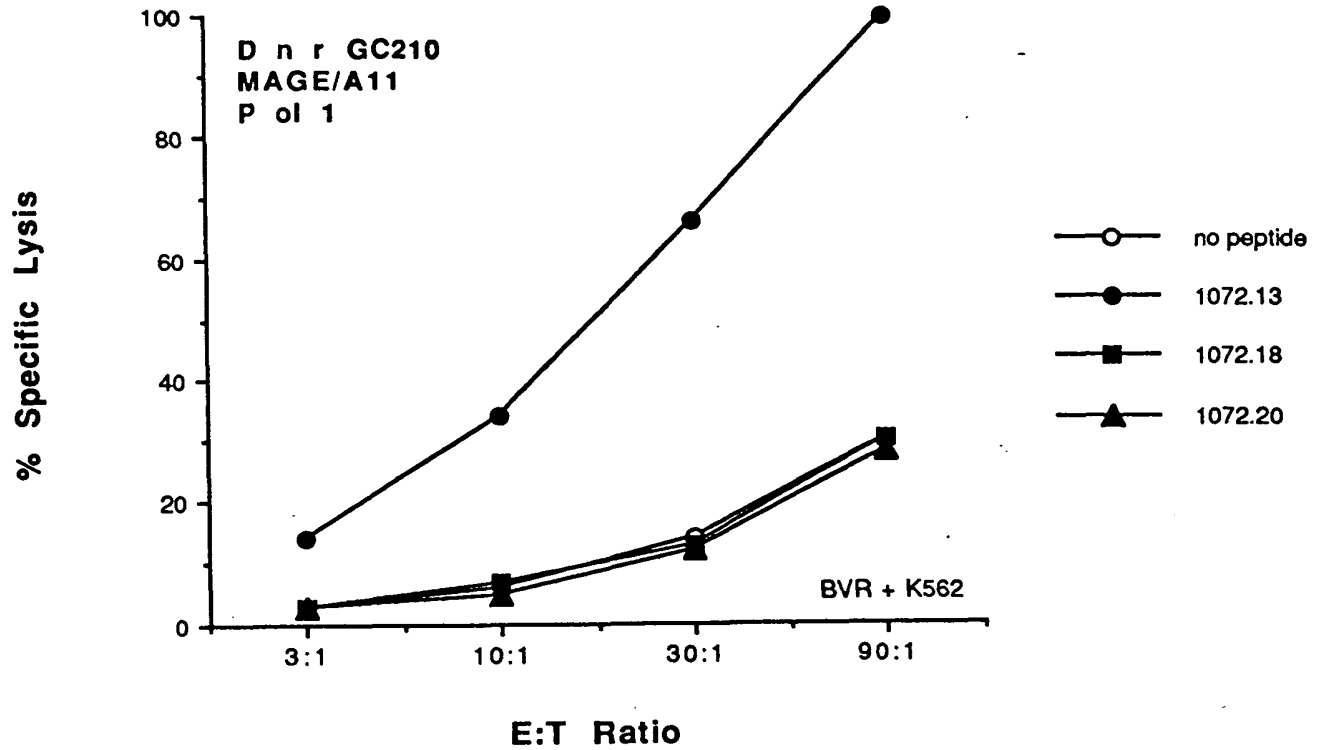


Figure 23

**Peptide Screening:
HIV/A3**

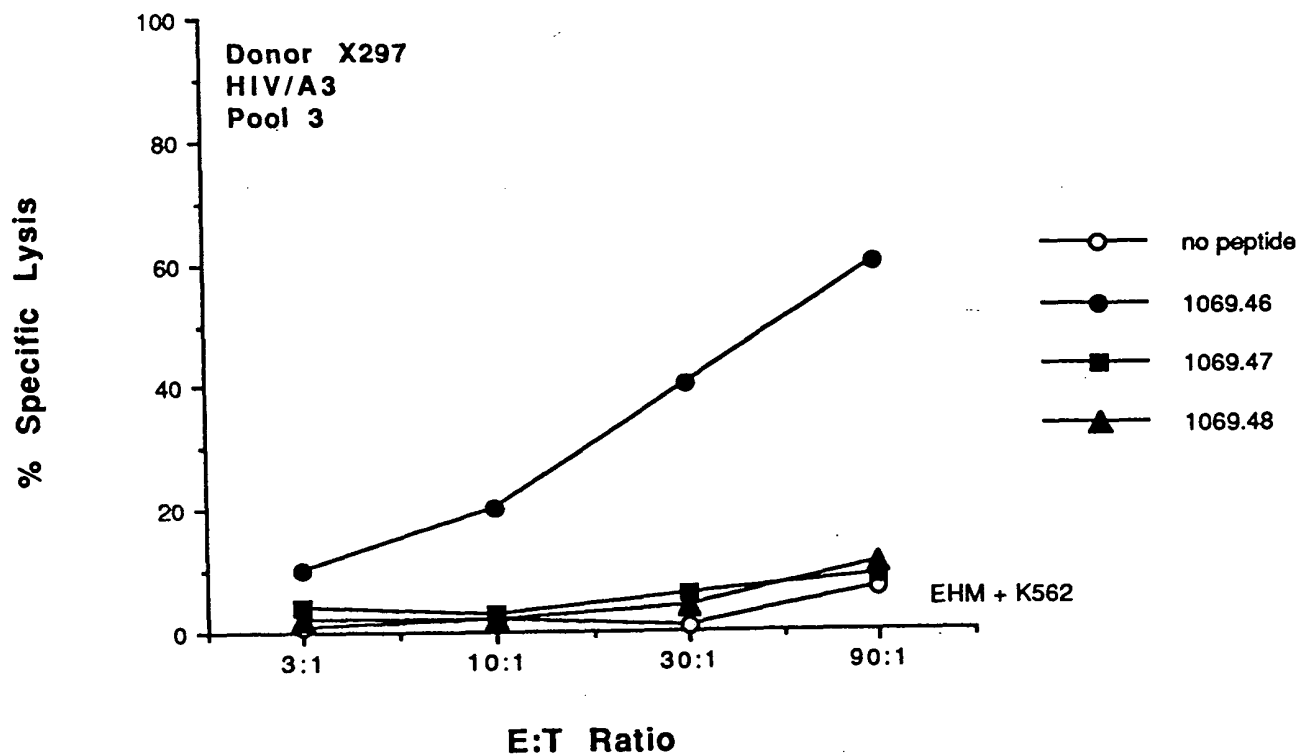
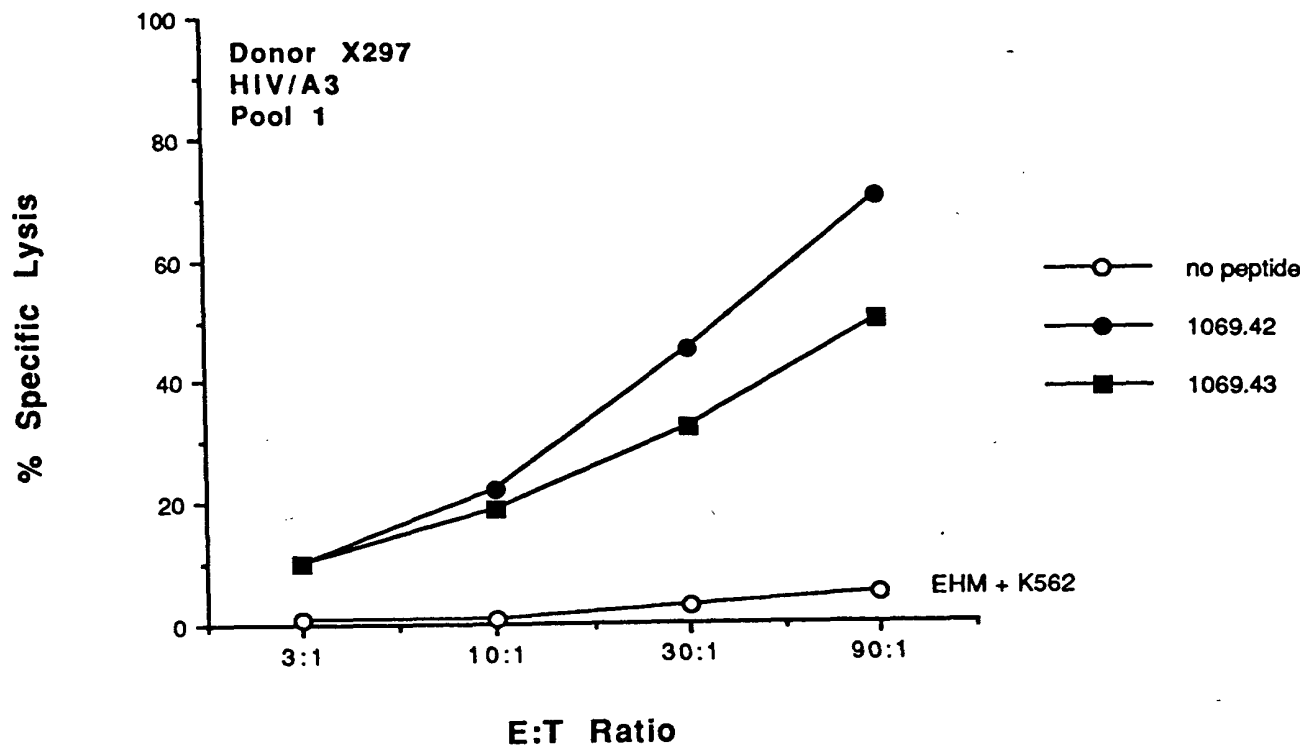


Figure 24

**Peptide Screening:
HCV/A3**

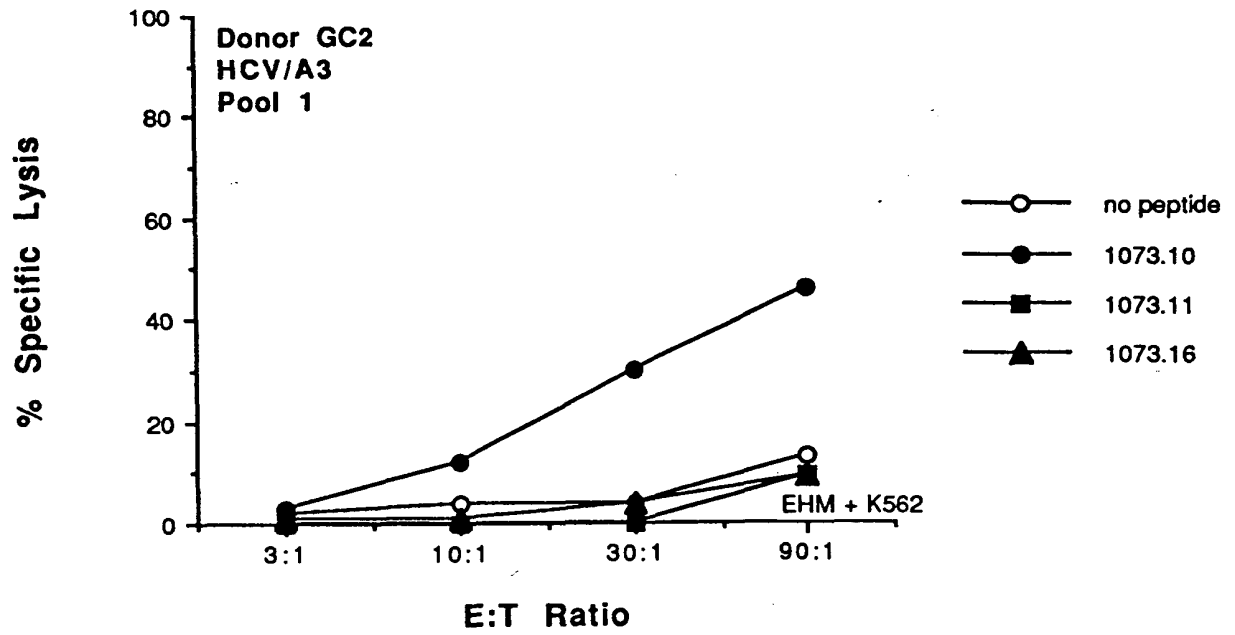


Figure 25

**Peptide Screening:
HBV/A3**

